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Vitamin A and Fat in the Herring *(Clupea harengus L.)* and in its Food

By L. R. FISHER, B.Sc., Ph.D. and ZENA D. HOSKING, B.A.

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By L. R. FISHER, B.Sc., Ph.D. and ZENA D. HOSKING, B.A.

National Institute for Research in Dairying, Shinfield, Reading
December, 1961

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Vitamin A and Fat in the Herring *(Clupea harengus L.)* and in its Food

INTRODUCTION

This investigation was undertaken to study the connexion between the biological characteristics and ıe vitamin A and fat contents of different organs in the herring, *Clupea harengus* L., and to determine ıe possible sources of the vitamin for this fish by analysing plankton and gut contents.

The project was planned jointly by the Marine Laboratory of the Department of Agriculture and isheries for Scotland, Aberdeen, the Oceanographic Laboratory, Edinburgh, and the Unit for Bio-ıemical Research bearing on Fisheries' Problems (grant-aided by the Development Fund) at the ational Institute for Research in Dairying, Shinfield.

The authors are grateful to Professor Sir Alister Hardy, F.R.S., Mr. B. B. Parrish, Mr. R. S. Glover, Ir. C. P. Cox and Dr. S. K. Kon for helpful criticism, and to Dr. C. E. Lucas for permission to publish ıe results of muscle fat determinations by Mr. B. A. Foster. Thanks are also due to Mr. G. W. Bonsall r statistical analysis of some of the larger groups of observations on the electronic computer at Rotham-ed Experimental Station, to Miss J. N. King and Miss B. Edwards for most of the laborious computing, ıd to other members of the laboratory staffs at Aberdeen, Edinburgh and Shinfield who carried out ıe bulk of the routine work. Help provided by the crews of Scottish research ships in collecting plankton ıd by Messrs. Explorator, Ltd. in transporting samples is also gratefully acknowledged.

The anatomical distribution of vitamin A in the herring has been previously studied by Edisbury *al* (1938) who found that most of the vitamin was concentrated in the pyloric caeca with appreciable mounts in the liver. The number of fish they analysed was, however, small and the authors pointed ıt that their results might not be representative of the main catches and that more tests were needed ı fish caught at different seasons and in different waters. In the investigations described in this paper, rgans have been examined from a large number of fish taken at frequent intervals during the Scottish orth-east coast fishery in the late spring and summer of 1953. These fish formed about 15% of the mples taken at Fraserburgh and described by Parrish *et al* (1954). In 1954 during the same fishery wer fish taken at less frequent intervals were analysed.

Other reports of extensive studies of vitamin A in the herring are those of Junker (1952-3) who bserved that the vitamin A concentration in the gonads increased with maturity up to spawning. Junker 956) further reported that the vitamin A concentrations in the flesh from herring collected in different reas of the North Sea bore no obvious relationship to the conditions prevailing in the area of collection. arlier work on vitamin A in herring has been concerned only with whole fish (Willstaedt & Jensen, 937; Holmes *et al*, 1941; Butler, 1946) or with samples or extracts from small numbers of organs (Schmidt-ielsen & Schmidt-Nielsen, 1925; Ahmad & Drummond, 1930; Schmidt-Nielsen *et al*, 1933; Lunde *et al*, 937; Lunde, 1938, 1938-9; Bacharach *et al*, 1942; Perepletshik, 1951; Braekkan & Probst, 1953; cheunart *et al*, 1956).

The present vitamin A determinations were made only at the Carr-Price reaction absorption ıaximum for vitamin A_1 of 618 mμ before Lambertsen & Braekkan (1956) showed that 15-30% of all ıe vitamin A in herring liver was vitamin A_2. Some absorption due to vitamin A_2 present would also ave been measured because, though the reaction product of vitamin A_1 shows little absorption at the 93 mμ maximum for vitamin A_2, the reaction product of vitamin A_2 absorbs appreciably at 618 mμ. he proportion of vitamin A_2 present cannot, however, be calculated, as the values for the 693 mμ ıaxima were not available. In this paper the term 'vitamin A' will, therefore, signify vitamin A_1 pre-ominantly, together with a variable proportion of vitamin A_2 when present in the extract.

Moreover, a comparison of the values for vitamin A in the gonads with those of Junker (1952-3) d to the discovery that vitamin A aldehyde was the predominant form in the ovaries (Fisher *et al*, 1956a;

Plack *et al*, 1959). Vitamin A aldehyde was not determined in any part of the herring for which results are now given so that the term 'vitamin A' is further qualified because it refers here only to the ester and alcohol forms together.

The study of variations in the fat content of organs of the herring most closely comparable to this work was made by Channon & El Saby (1932) during a season of the Isle of Man fishery. Wood (1958) measured the fat content of whole North Sea herring and referred to earlier work on fat in this species but not to that of Junker (1956) on muscle from fish caught in the North Sea and that of Leim (1957, 1958) on whole fish caught in Canadian Atlantic waters.

MATERIAL AND METHODS

Plankton Sampling

Plankton collections were made in 1953 at about 3-weekly intervals, except in June and July when the ship broke down. All the samples were taken by vertical hauls from the bottom to the surface with the Hensen or the 1-metre silk net. In 1954 the sampling covered a wider area on the fishing ground than in the previous year. On this occasion oblique hauls were made with the 1-metre silk net. Details are given in Table I.

TABLE I

THE AREAS FROM WHICH PLANKTON SAMPLES WERE COLLECTED

Date of sampling	Number of stations	Area
4-7 May 1953	25*	57°15′N to 58°25′N × 0°20′E to 1°50′W
25-29 May 1953	23	57°15′N to 58°25′N × 0°20′E to 1°50′W
3-7 Aug 1953	11	57°15′N to 57°55′N × 0°00′ to 1°50′W
17-20 Aug 1953	14	57°15′N to 58°25′N × 0°20′E to 1°43′W
19-24 Apr 1954	16	57°00′N to 58°15′N × 2°00′E to 1°30′W
17-18 May 1954	13	56°15′N to 57°00′N × 1°00′E to 2°30′W
17-20 May 1954	17	57°45′N to 58°30′N × 2°00′E to 2°00′W
29 Jun—3 Jul 1954	17	57°15′N to 58°15′N × 2°00′E to 2°00′W
2-10 Aug 1954	18	57°00′N to 58°15′N × 2°00′E to 2°00′W

*4 of these samples contained insufficient material for analysis.

Herring Sampling

Fifty adult herring were taken on 17 occasions at approximately weekly intervals from 8 May to September 1953, from catches by commercial drifters operating from Fraserburgh. The herring were selected at random from randomly-chosen vessels according to the sampling methods described by Parrish (1956), who demonstrated that 50 herring taken in this way from the top of the catch in the ship's hold provide a reasonably unbiassed sample of the catch of that vessel. Only autumn-spawned herring were considered in this investigation, spring-spawned fish, which constituted fewer than 2·5% of the 85 fish sampled, being discarded.

Sampling in 1954 was selective. Five adult herring were taken on each of eight occasions from 1 May to 21 August. Crews of two commercial drifters selected seven or eight fish from the decks of the vessels as soon as the nets were hauled, measuring sticks being used to obtain fish of 26·0 to 26·9 cm in length. The fish were then stored in kipper boxes to ensure no loss of gut contents by pressure. When the boats returned to Fraserburgh, a member of the staff of the Edinburgh Oceanographic Laboratory selected five fish with food in their caeca from those in the boxes.

Ten female and ten male 1-group and forty-two 0-group herring were selected by Mr. J. H. S. Blaxter of the Marine Laboratory, Aberdeen, during the Moray Firth fishery in December 1954.

Post-sampling Treatment of Herring

In 1953 the herring were dissected on the day on which they were collected from the boats. After standard measurements of length and weight had been taken, the alimentary canal, liver (including gall-bladder), gonads and eyes were removed from each herring and the contents taken from the oeso

phageal caecum. Liver colour was determined subjectively and noted as dark-red, red, mottled or variegated, or yellow. The organs were set aside for vitamin A determination together with the caecal contents from 25 of each lot of 50 fish. Caecal contents from the remaining fish were analysed at the Oceanographic Laboratory, Edinburgh, for the species present in the food. The carcases were retained by the Marine Laboratory, Aberdeen, for otolith analysis, vertebral counts and other determinations, whilst muscle from the posterior half of the fish on one side of the vertebral column was removed for fat analysis.

In 1954 also each adult herring was dissected on the day of sampling. After first plugging the anus and ligating the gut to prevent the escape or transfer of the gut contents, the alimentary canal was divided into oesophagus and caecum, pylorus, pyloric caeca, proximal intestine and distal intestine and, when possible, the contents of the different parts were removed from the lumen and preserved separately. The demarcation between the oesophageal caecum and the pylorus was the point, about 6 mm from the branch of the caecum, at which the thin-walled gut became muscular and thick-walled, and that between the proximal and distal parts of the intestine was where the intestine passed the end of the caecum as it lay in the body cavity. In addition, the liver, with gall-bladder, and the mesenteric fat were kept for analysis. For 1-group herring, the pyloric and intestinal parts of the alimentary canal, the oesophageal caecum with contents and the liver were removed for vitamin A determinations. The 0-group herring were preserved separately as whole specimens.

Preservation and Analysis of Material

All the material was placed in glass containers, preserved in 70% ethanol and kept in cold store at Aberdeen. Samples for vitamin A analysis were sent to Shinfield a few days later, by insulated fish lorry in 1953 and by passenger train in 1954. At Shinfield they were kept at $-20°C$ until analysed.

The ethanol was drained off from the organs, which were weighed to give an approximate wet weight. The dry weights of the alimentary canal contents and of the plankton were determined by extracting the fat with light petroleum (b.p. 40-60°C) in a Waring Blendor (Fisher *et al*, 1952) and drying the fat-free residue to unchanging weight in a hot air current at about 60°C.

The method of analysis to determine vitamin A and carotenoids was basically that of Fisher *et al* (1952) with slight modifications for the different materials. Preliminary analysis showed that nearly all the vitamin A in the liver was in the ester form and that there was very little vitamin A in the eyes. For these organs, therefore, the presaponification chromatography was omitted in the 1953 investigation. The non-saponifiable residues from the eyes and livers contained no pigments and so the second chromatography was also dispensed with. In 1953, the plankton, caecal contents, alimentary canals and gonads, and in 1954 the plankton, contents and walls of all parts of the alimentary canals and the livers, all parts of the 1-group and whole specimens of the 0-group fish were subjected to the full analytical procedure with chromatography both before and after saponification.

The fat in the muscle fillets was determined by Mr. B. A. Foster at the Marine Laboratory, Aberdeen. The muscle was first macerated in an Ato-Mix blender with the ethanol in which it had been preserved, together with diethyl ether washings of the bottle. Water was added, if necessary, to make up the level of liquid in the blender. The homogenate was transferred to a dish, dried in an oven at 80°C for 18 hours and then weighed. The dried sample was transferred to a filter paper thimble and extracted with diethyl ether in a soxhlet apparatus, diethyl ether rinsings from the dish being included in the extraction liquids. The redried dish was weighed and the dry weight of the sample obtained by subtraction. After extraction for six hours the oil from the sample was collected in the soxhlet flask and weighed after removal of the ether by gentle heating under reduced pressure.

In both 1953 and 1954 the analytical results from some fish were incomplete because of accidental loss of particular organs or measurements.

Since the amount of fat in some organs varied greatly during the season, concentrations of vitamin A and fat have been expressed in terms of the fat-free tissue of the organ rather than in terms of the fat or whole tissue as is the more usual practice. Concentrations in plankton and in gut contents are given in terms of the dry weight of fat-free tissue, whereas concentrations in the wall of the alimentary canal and in the other organs are based on the wet weight of the fat-free tissue. When it seemed particularly

appropriate, for example for the liver and the gonads, the absolute amount of vitamin as well as its concentration in the organ was studied.

The fat content of muscle was originally expressed in terms of the weight of dry tissue. Since, however, the protein content of herring is constant for practical purposes at 20% and fat and water constitute, in relatively variable proportions, the remaining 80% of the fresh weight (Foster, 1954), these relationships have been used to express fat as a percentage of wet weight of fat-free tissue.

The distributions of the observed concentrations of vitamin A and fat in the different organs of the herring were markedly skew. After logarithmic transformation of the results, much more symmetrical distributions were obtained for which departures from normality were not serious. Typical examples of the distributions obtaining in observed and transformed results are shown in Figs. 9 and 10 for the concentration of vitamin A in the liver.

Mean values for vitamin A, carotenoids and fat given in the text have been derived from the corresponding means of the logarithmically transformed data. Geometric means of the observed values were calculated for plankton, 1-group and 0-group herring, and also for the overall results for the different observations made on the food, organs and part-organs of the adult herring. The derived mean contents and concentrations of vitamin A and fat in the organs of the adult herring of different ages, maturities and sexes were obtained directly from the unbiassed means estimated from fitted constants.

Inspection of the data obtained from the 1953 fish suggested that pooling the values from the older herring would not cause serious loss of information and in all the analyses of variance, therefore, all fish with more than 9 rings have been included in the 10-ring age group. When the results were classified according to two sexes, nine ages and seven maturities it was found that there were at least 30 sub-classes with no results, that about 25 had one value and that about 10 had observations from at least 20 fish: values for 4-ring and older fish at stages I, III and IV were extremely few and all ages were only sparsely represented at maturities V, VI and VII. Analyses of variance by fitting constants were carried out on the transformed values to examine the effects of sex, age and maturity on the content and concentration of vitamin A and fat in most of the organs taken from adult herring sampled in 1953. The residual mean square after fitting age, maturity and sex was not significantly larger than that for within sub-classes ($P > 0{\cdot}05$) in any analysis of variance other than for the amount and concentration of vitamin A in the liver. Examination of the observed mean values of vitamin A in the liver of male and female fish at different maturities indicated that the changes with maturity were not the same for both sexes and, consequently, the results for males and females were analysed separately. In other analyses of variance, the age and maturity differences in the weight of the fat-free liver tissue of male and female fish and the sex and maturity differences in vitamin A in the gonads of fish of all ages were examined.

Among the 1954 adult herring, as there was only one fish with 5 rings and none older, results from the 5-ring fish were pooled with those from the 4-ring fish. No spent fish (stage VII) were obtained. The effects of sex, age and maturity on the content and concentration of vitamin A and fat in the organs or part-organs of the herring were studied and analyses of variance by fitting constants were carried out on the transformed values of vitamin A and fat. Similar analyses were made on the logarithms of the fat concentrations in the food taken from different parts of the intestine, but not on the vitamin concentration because vitamin A was not found in many of the food samples examined.

Analyses of variance were also carried out on the transformed concentrations of vitamin A and fat in the organs of 1-group herring taken in December 1954, differences between the young male and female fish being of interest.

Regressions were calculated to obtain information on the relationship of vitamin A and fat to the total body weight of 0-group herring caught in December 1954.

GENERAL CHARACTERISTICS OF THE HERRING STUDIED

Adult Herring Collected in 1953

The lengths of 805 fish ranged from 22·1 to 31·7 cm and the weights from 89 to 290 g. The proportion of male to female fish was 0·98:1·00, the average lengths being 26·4 and 26·3 cm and the corresponding mean weights 158 and 155 g respectively.

Age was generally determined from the number of rings on the otolith, only infrequently from the rings on the scale. Ring-age varied from 2 to 16 with 72% of the fish having 2 or 3 rings and only 5% having 9 or more. The age distribution of the herring collected at different times is shown in Fig. 1 and the mean lengths and weights at different ages are given in Table II.

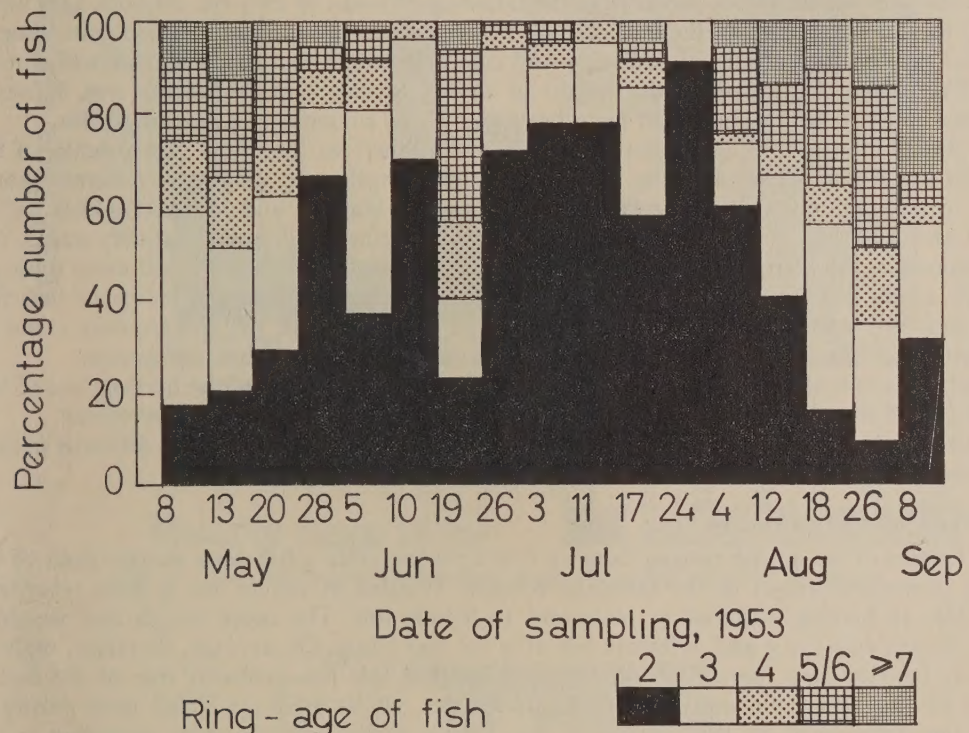

Fig. 1 The age distribution of herring collected at different times in 1953.

TABLE II

THE MEAN LENGTH AND WEIGHT OF FISH OF DIFFERENT AGES AND MATURITIES

	Herring taken in 1953			Herring taken in 1954		
Ring-age	Number of fish	Length (cm)	Weight (g)	Number of fish	Length (cm)	Weight (g)
2	369	24·9	136	17	26·4	165
3	209	26·6	158	18	26·6	172
4	62	27·1	168	4	26·4	152
5	57	28·6	190	1	26·9	183
6	45	28·2	187	0	—	—
⩾7	63	29·4	208	0	—	—
Maturity stage						
I	182	24·5	124	5	26·5	145
II	264	26·3	147	14	26·5	156
III	100	25·7	154	7	26·5	173
IV	97	26·6	177	7	26·4	177
V	64	28·6	208	5	26·6	189
VI	47	28·5	205	2	26·0	181
VII	51	28·7	174	0	—	—
All fish	805	26·3	156	40	26·5	167

Seasonal trends in the lengths and weights of 2- and 3-ring fish were observed. From May to July the 3-ring fish averaged about 26 cm in length and increased to nearly 28 cm from mid-August to September. Results for 2-ring fish were similar except that some unusually small herring were taken in two mid-season catches and in the final one; the average length in September was about 25 cm and similar to the pre-August values rather than the late-August mean of 26·5 cm. At both ages body-weight tended to increase slowly up to the end of July, followed by a more rapid increase coinciding with the appearance of high proportions of near-ripe and ripe fish and then a sharp decline with spents in the sample. The observed decrease in the weight of 2-ring fish taken in September was, however, more marked than for the 3-ring and could have been due to the presence of a few small fish.

The distribution according to maturity stage, determined on the Hjort (1910) scale, of fish taken throughout the season is shown in Fig. 2, and the mean lengths and weights at different maturities in Table II. Approximately 55% of the fish examined were at stages I and II whereas only 12% were at stages VI and VII. Fig. 3 shows the age distribution of herring at different maturity stages. Nearly all the 182 immature fish were in the 2- or 3-ring age groups and fewer than 2% had more than 4 rings.

In the 2-ring fish the seasonal relationship between maturity and length indicated that the longer fish had generally reached a more advanced stage of maturity than the shorter ones taken from the same catch. Small numbers of fish precluded useful comparisons for other age groups.

Vertebral counts of 779 herring were recorded. Approximately 42% of the herring had 56 vertebrae, 47% had 57 and the remaining fish, excepting one with 59, had either 55 or 58 vertebrae.

No attempt has been made to analyse separately the results obtained during different periods of the fishing season when different races of herring may have been sampled.

Adult Herring Collected in 1954

The lengths of the 40 fish ranged from 25·2 to 27·0 cm; only 3 fish were shorter than 26·0 cm, the minimum prescribed length in the sampling scheme. Weights of 125 to 202 g were recorded for the herring. The 40 herring included 21 male and 19 female fish. The mean length and weight of male fish was 26·5 cm and 172 g and of female fish 26·4 cm and 162 g. On average, therefore, male fish were appreciably heavier than female fish of the same size but this was probably due to the fact that the immature fish in the sample were predominantly females, whilst the stage V fish were mostly males.

Age was determined by the number of rings on the otolith and maturity according to the Hjort (1910) scale. The numbers, mean lengths and weights of the herring of different ages and maturities are given in Table II.

Vertebral counts were recorded from 31 fish of which nine had 56 vertebrae, nineteen had 57 and three had 55 or 58.

Comparison of the Adult Herring taken in 1953 and 1954

Bearing in mind that these are biassed comparisons because of the highly selective sampling in 1954, the 2- to 5-ring fish taken in 1953 with a mean length of 25·9 cm and a mean weight of 150 g were shorter by 0·6 cm and lighter by 17 g than herring of the same age in 1954.

The normal relationship of increasing length with increasing age was clearly indicated in 1953 but, naturally, was not obtained in 1954 when the lengths of the fish sampled were virtually constant at all ages. Increase in weight with advancing maturity, though more marked in 1953, when the fish taken at the later maturity stages were generally longer than those at the earlier stages, was also evident in 1954, when differences between the mean lengths of herring at the various stages were trivial (Table II).

RESULTS

Plankton

Dry weights of the 69 plankton samples analysed in 1953 ranged from 3·5 to 3435 mg, including 12 samples of under 200 mg and 12 of over 1000 mg. Vitamin A was not detected in any sample and, even after the residues from all samples had been combined and concentrated, the Carr-Price reaction was negative.

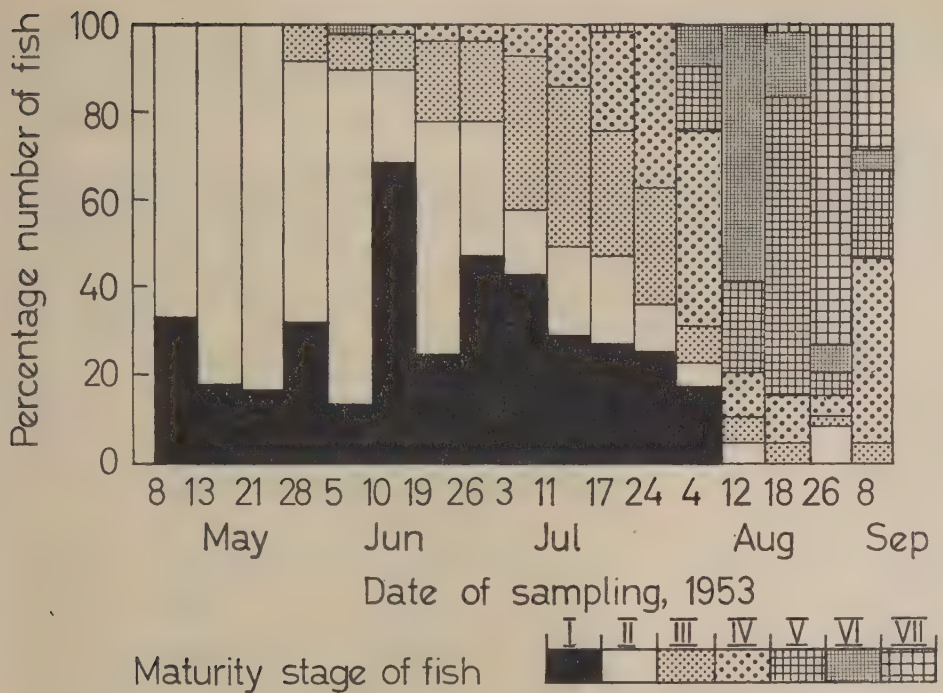

Fig. 2 The distribution by maturity stage of herring collected in 1953.

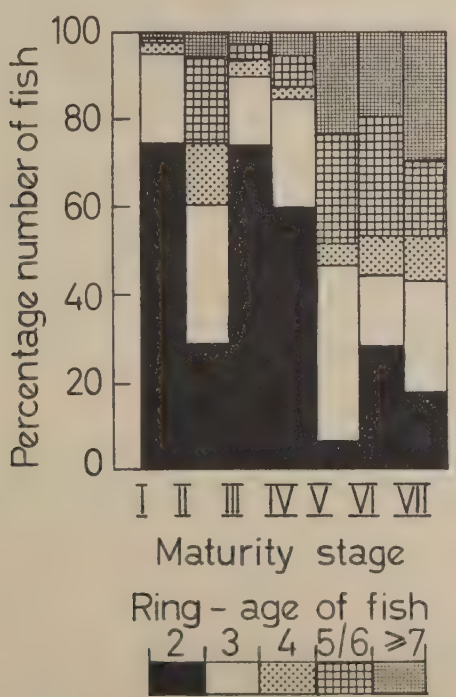

Fig. 3 The age distribution of fish taken at different maturity stages during 1953.

The carotenoids present in the plankton samples for 1953 were mainly xanthophylls or astaxanthin, and β-carotene was found only in trace amounts. Mean values for carotenoid concentrations in the plankton samples taken on each cruise are given in Table III. The overall mean was 49·0 μg/g dry, fat-free tissue and individual concentrations ranged from 0 to 420 μg/g with 6 values of either under 10 or over 200 μg/g.

Mean fat concentrations obtained in the plankton samples taken at different times in 1953 also appear in Table III. Concentrations in the 69 samples averaged 14·8 g/100 g and varied from 0·8 to 117 g/100 g dry, fat-free tissue.

TABLE III

VITAMIN A, CAROTENOIDS AND FAT IN THE PLANKTON COLLECTED IN 1953 AND 1954

Date of sampling	Number of samples	Number with vitamin A	Mean vitamin A concentration (μg/g dry, fat-free tissue)	Mean carotenoid concentration (μg/g dry, fat-free tissue)	Mean fat concentration (g/100g dry, fat-free tissue)
4-7 May 1953	21	0	—	42·2	17·5
25-29 May 1953	23	0	—	36·7	13·3
3-7 Aug 1953	11	0	—	55·1	7·4
17-20 Aug 1953	14	0	—	90·2	16·0
19-24 Apr 1954	19*	18	1·34	292	31·7
17-18 May 1954	13	10	0·39	79·3	18·5
17-20 May 1954	17	16	0·70	266	29·3
29 Jun—3 Jul 1954	17	15	0·44	141	13·0
2-10 Aug 1954	21*	17	0·43	318	21·4

*Including 3 large samples, each analysed in 2 parts.

Much larger samples of plankton were obtained in 1954 when collection by oblique hauls replaced the vertical hauls of 1953. Dry weights of the samples ranged from 292 to 14082 mg but only seven of the 87 samples weighed less than 1000 mg, and ten samples exceeded 10000 mg. When the samples were so large as to necessitate dividing them into two parts for storage, the parts were analysed separately.

Vitamin A was present in 76 out of 87 samples (Table III). The mean value for the 87 samples was 0·64 μg/g dry, fat-free tissue with concentrations ranging from 0·1 to 6·5 μg/g in those samples in which vitamin A was detected.

The concentration of carotenoids in the plankton samples collected in 1954 was much higher than in those of 1953 even though the main components were the same. The mean value for the 87 samples was 198 μg/g dry, fat-free tissue and individual results ranged from 3·7 to 1960 μg/g.

Mean fat concentrations in the 1954 samples are also shown in Table III. The mean value for the season was 22·2 g/100 g dry, fat-free tissue and individual concentrations varied from 2·4 to 65 g/100 g.

The 1953 plankton samples were inspected only superficially for the types of organisms whereas those of 1954 were examined more closely. The main animal groups in 1954 are listed in Table IV.

TABLE IV

THE OCCURRENCE OF DIFFERENT ANIMAL GROUPS IN THE 87 PLANKTON SAMPLES TAKEN IN 1954 AND THE FREQUENCY WITH WHICH VITAMIN A WAS FOUND

Planktonic animals	Samples in which animals were present		
	Number of samples	Number with vitamin A	Percentage with vitamin A
Copepods	68	60	88
Euphausiids	36	33	92
Chaetognaths	9	6	67
Fish larvae and *Ammodytes*	7	5	71
Coelenterates and ctenophores	4	4	100
Mysids	4	3	75
Gastropods	2	2	100
Unidentified	11	9	82

together with the frequency with which vitamin A was found in the samples containing the particular groups. Glover (1957) has given a more detailed account of the composition of the plankton in the same area in the two years.

Contents of the Oesophagus and Caecum

The food of the herring taken in 1953 is shown in Fig. 4, which was prepared at the Oceanographic Laboratory, Edinburgh, after the caecal contents from 25 of the 50 fish sampled on each occasion had been identified and counted. Scales of the histograms in Fig. 4 conform with Savage's (1937) modification of Hardy's (1924) proposal that representations of differently-sized organisms should be weighted to make them similar on a food value basis: in Fig. 4, therefore, young fish have been rated at 100 units, euphausiids and decapod larvae at 15 and other organisms at unit value.

Contents of the oesophageal caeca from the remaining 25 fish of each collection were analysed for vitamin A, but the species present were not identified because prolonged illumination of the material for microscopic examination might have caused destruction by light of vitamin A. Tables V and VI

TABLE V

VITAMIN A IN THE CONTENTS OF THE OESOPHAGUS AND CAECUM OF HERRING COLLECTED IN 1953

Date of sampling	Number of herring	Number with contents	Number with vitamin A in contents	Concentrations of vitamin A when present in contents (μg/g dry, fat-free tissue)
8 May	25	25	8	2·79-8·61
13 „	24	22	15	5·20-68·2
20 „	25	19	3	10·1-16·0
28 „	25	24	9	5·75-23·6
5 Jun	25	20	2	5·20-12·1
10 „	25	23	10	2·54-270
19 „	25	23	3	4·12-7·71
26 „	25	20	4	2·17-605
3 Jul	24	12	1	17·7
11 „	25	23	9	3·44-9·36
17 „	25	19	3	4·45-127
24 „	21	4	2	21·9-62·8
4 Aug	25	4	0	—
12 „	25	1	0	—
18 „	24	5	2	17·8-49·0
26 „	25	11	1	16·8
8 Sep	24	2	0	—
Total	417	257	72	2·17-605

show the results for the fish sampled in 1953 and in 1954. The limited comparisons possible suggest that the two sets of observations are in general agreement. The mean vitamin A concentration in the contents of this part of the gut is shown in Fig. 5.

Fat concentration was determined in the caecal contents from 248 herring collected in 1953. The mean concentration was 50·3 g/100 g dry, fat-free tissue with 95% confidence limits of 45·8 and 55·4 g/100 g. Sex differences were very small; age differences, though greater, were not statistically significant. Maturity effects on fat concentration were, however, significant ($P < 0{\cdot}05$). The mean values at maturity stages I to VII were 57·1, 54·4, 39·5, 38·2, 48·3, 51·2 and 22·3 g/100 g respectively and they illustrate the general tendency for fat concentration to decline with advancing maturity. The mean fat concentration in the contents of the oesophageal caeca of 27 herring sampled in 1954 is given in Table VII (see also Fig. 6): no significant effects due to sex or age and maturity were obtained.

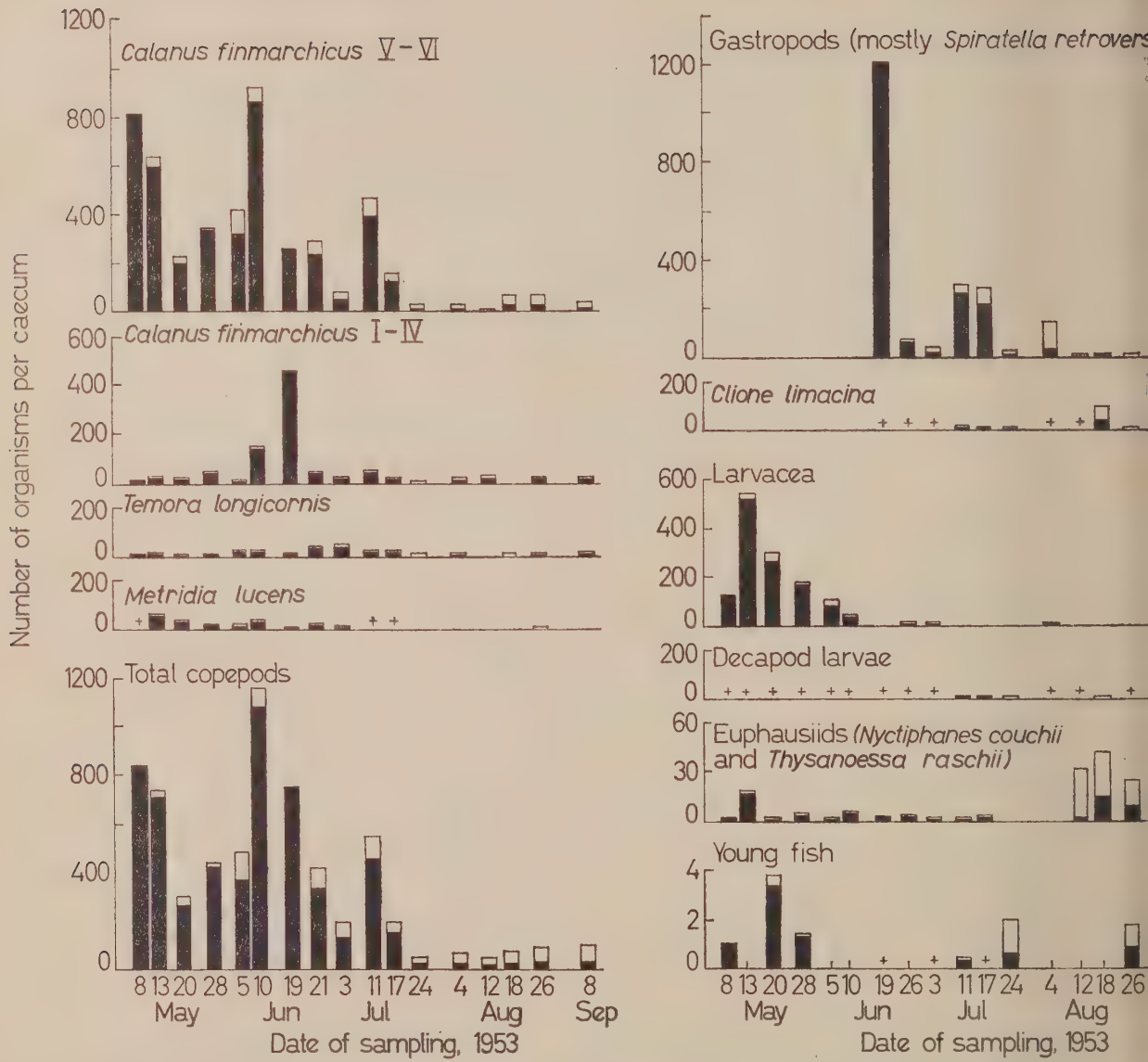
Number of organisms per caecum
Calanus finmarchicus V-VI
Calanus finmarchicus I-IV
Temora longicornis
Metridia lucens
Total copepods
Gastropods (mostly Spiratella retrovers
Clione limacina
Larvacea
Decapod larvae
Euphausiids (Nyctiphanes couchii and Thysanoessa raschii)
Young fish
8 13 20 28 5 10 19 21 3 11 17 24 4 12 18 26 8
May Jun Jul Aug Sep
Date of sampling, 1953
8 13 20 28 5 10 19 26 3 11 17 24 4 12 18 26
May Jun Jul Aug
Date of sampling, 1953

Fig. 4 The food of herring taken in 1953: mean values for feeding herring denoted by whole column ▬, and mean values over all fish by ■.

TABLE VI

VITAMIN A IN THE CONTENTS OF DIFFERENT PARTS OF THE ALIMENTARY CANALS OF HERRING COLLECTED IN 1954: 5 ALIMENTARY CANALS EXAMINED AT EACH SAMPLING

Date of sampling	Number with contents	Number with vitamin A in contents	Concentration of vitamin A when present in contents (μg/g dry, fat-free tissue)
		Oesophagus and caecum	
14 May	5	5	2·30-10·7
21 May	4	0	—
17 Jun	5	1	53·2
30 Jun	4	1	18·0
16 Jul	5	1	467
30 Jul	2	0	—
6 Aug	0	0	—
21 Aug	2	1	42·0
Total	27	9	2·30-467
		Pylorus	
14 May	5	0	—
21 May	3	1	+
17 Jun	4	1	+
30 Jun	4	1	143
16 Jul	4	0	—
30 Jul	3	0	—
6 Aug	2	0	—
21 Aug	1	0	—
Total	26	3	143
		Pyloric caeca	
14 May	5	5	13·1-71·4
21 May	1	0	—
17 Jun	5	4	4·84-58·7
30 Jun	4	0	—
16 Jul	5	2	36·2, 55·7
30 Jul	5	2	27·0, 27·0
6 Aug	2	0	—
21 Aug	4	0	—
Total	31	13	4·84-71·4
		Proximal intestine	
14 May	5	1	37·8
21 May	2	1	25·8
17 Jun	5	0	—
30 Jun	5	1	43·9
16 Jul	5	2	38·8, 71·9
30 Jul	5	0	—
6 Aug	4	0	—
21 Aug	5	2	111, 441
Total	36	7	25·8-441
		Distal intestine	
14 May	4	1	25·8
21 May	4	3	21·4-127
17 Jun	5	2	20·0, 28·9
30 Jun	5	2	29·9, 56·0
16 Jul	3	0	—
30 Jul	5	0	—
6 Aug	3	0	—
21 Aug	5	0	—
Total	34	8	20·0-127

+ Vitamin A known to be present but weight of fat-free tissue not determined.

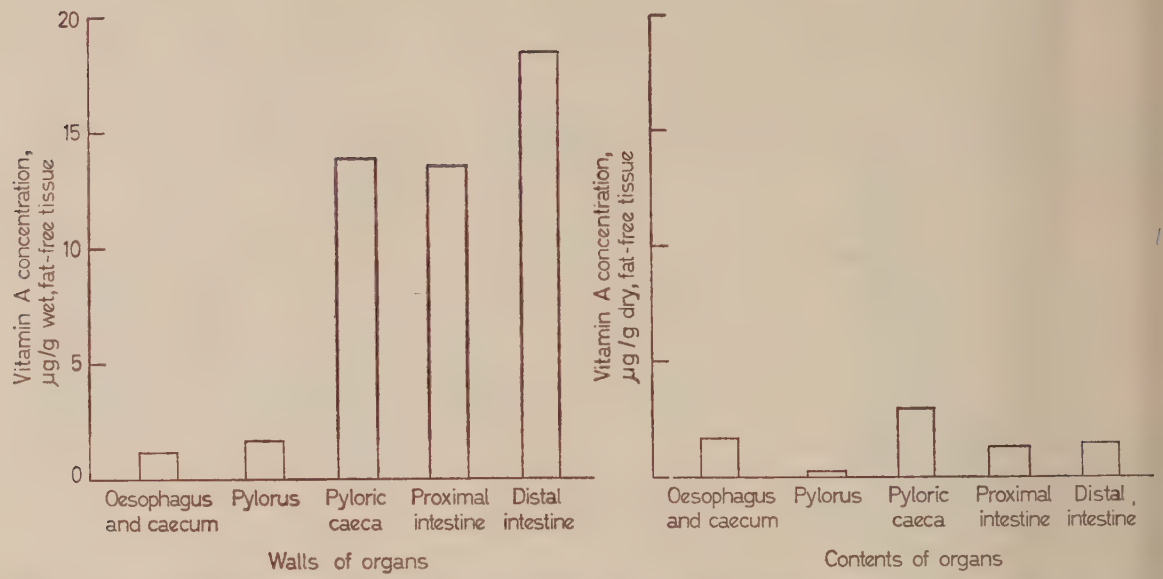

Fig. 5 The mean concentration of vitamin A in the wall and in the contents of different parts of the alimentary canal of herring collected in 1954.

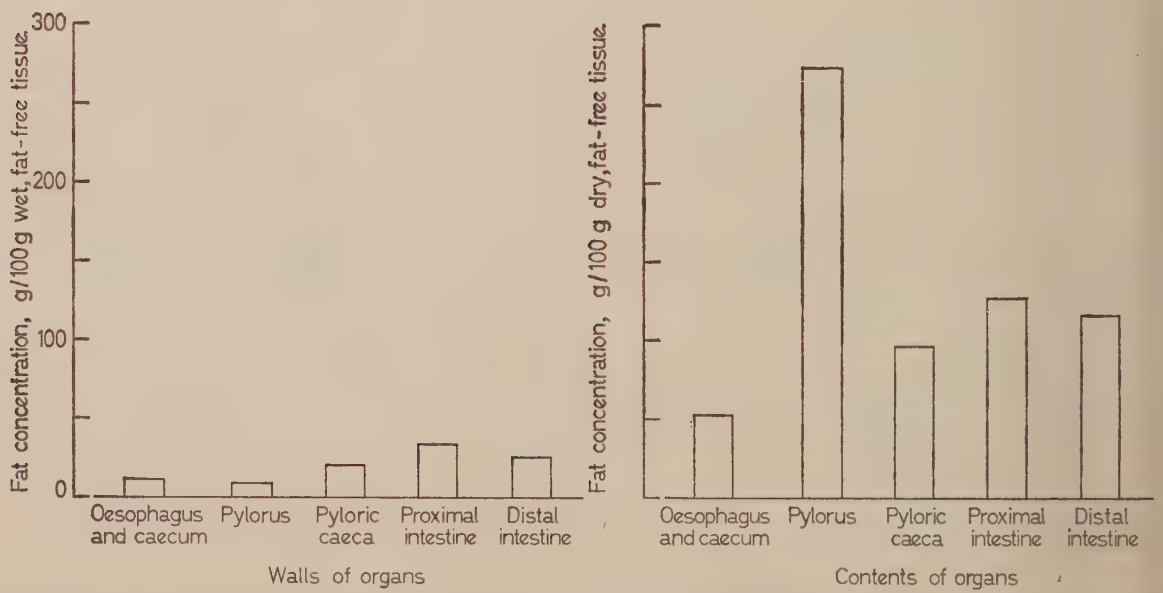

Fig. 6 The mean concentration of fat in the wall and in the contents of different parts of the alimentary canal of herring collected in 1954.

ONTENTS OF THE PYLORIC AND INTESTINAL GUT

Food was sometimes absent from parts of the alimentary canal and when present did not always ontain vitamin A in measurable quantities. Vitamin A was not isolated from the contents of all parts of e gut of any fish.

The occurrence of vitamin A was rather variable between different organs from fish taken at the me time and between similar organs from herring sampled on different dates (Table VI). There are sufficient data to determine whether any general change in the concentration of vitamin A occurs uring passage along the canal. Mean values of vitamin A concentrations in the contents from each art of the gut from all the adult herring taken in 1954 are shown in Fig. 5.

TABLE VII

FAT CONCENTRATIONS (g/100g DRY, FAT-FREE TISSUE) IN THE CONTENTS OF DIFFERENT PARTS OF THE ALIMENTARY CANAL: FISH TAKEN IN 1954

	Oesophagus and caecum	Pylorus	Pyloric caeca	Proximal intestine	Distal intestine
umber of fish	27	22	30	35	33
lean concentration	52·9	270	96·4	115	124
5% confidence limits of mean	27·4, 101	107, 680	68·1, 136	70·4, 186	73·4, 210

Fat concentrations in nearly all the contents obtained from different parts of the alimentary canal were etermined. Mean values are given in Table VII and Fig. 6. The fat concentration in the contents of e pylorus was appreciably higher than in the contents of the other parts of the gut. The effects of age nd maturity on the fat concentration were not significant, nor was the effect of sex except in the distal testine which averaged 286 and 45 g/100 g dry, fat-free tissue for male and female fish respectively.

TABLE VIII

MEAN VALUES OF VITAMIN A AND FAT IN THE WALL OF THE ALIMENTARY CANAL OF HERRING COLLECTED IN 1953 AND 1954

Ring-age	Vitamin A concentration (μg/g wet, fat-free tissue)		Vitamin A amount (μg)	Fat concentration (g/100 g wet, fat-free tissue)	
	1953	1954	1953	1953	1954
2	12·9	7·65	67·3	27·0	29·2
3	12·7	11·7	71·3	21·6	24·7
4	16·4	15·9	90·7	20·2	43·1
5	15·5	—	95·0	17·9	—
6	15·0	—	93·6	16·4	—
7	16·3	—	113	14·0	—
8	10·9	—	70·1	16·8	—
9	11·7	—	40·5	13·7	—
⩾ 10	12·3	—	86·4	9·77	—
Maturity stage					
I	13·0	6·79	72·9	40·5	37·7
II	12·0	9·10	79·8	31·5	34·2
III	17·1	21·1	96·8	30·0	53·0
IV	14·9	10·6	71·9	14·6	21·7
V	13·2	11·9	60·1	6·87	18·3
VI	14·8	13·2	54·8	5·58	6·52
VII	12·1	—	51·7	6·08	—
Total fish	768	30	749	775	30
Mean value	13·4	10·6	73·7	22·1	28·1
95% confidence limits of mean	12·6, 14·2	7·09, 15·7	69·7, 78·0	21·0, 23·3	23·7, 33·3

Wall of the Alimentary Canal

Entire gut

Table VIII shows mean values of vitamin A concentration in the wall of the alimentary cana from fish of different ages and maturity stages collected in 1953 (see also Fig. 7). There was no significan difference between the sexes or between age groups ($P > 0{\cdot}05$) but there was some evidence of rea differences ($P < 0{\cdot}05$) between the vitamin A concentrations obtaining at the different maturity stages.

Results were obtained for the entire gut from only 30 fish in 1954, and mean vitamin A concentration are shown in Table VIII and in Fig. 7. The analysis of variance indicated that the effects of sex, age an maturity were not significant ($P > 0{\cdot}05$).

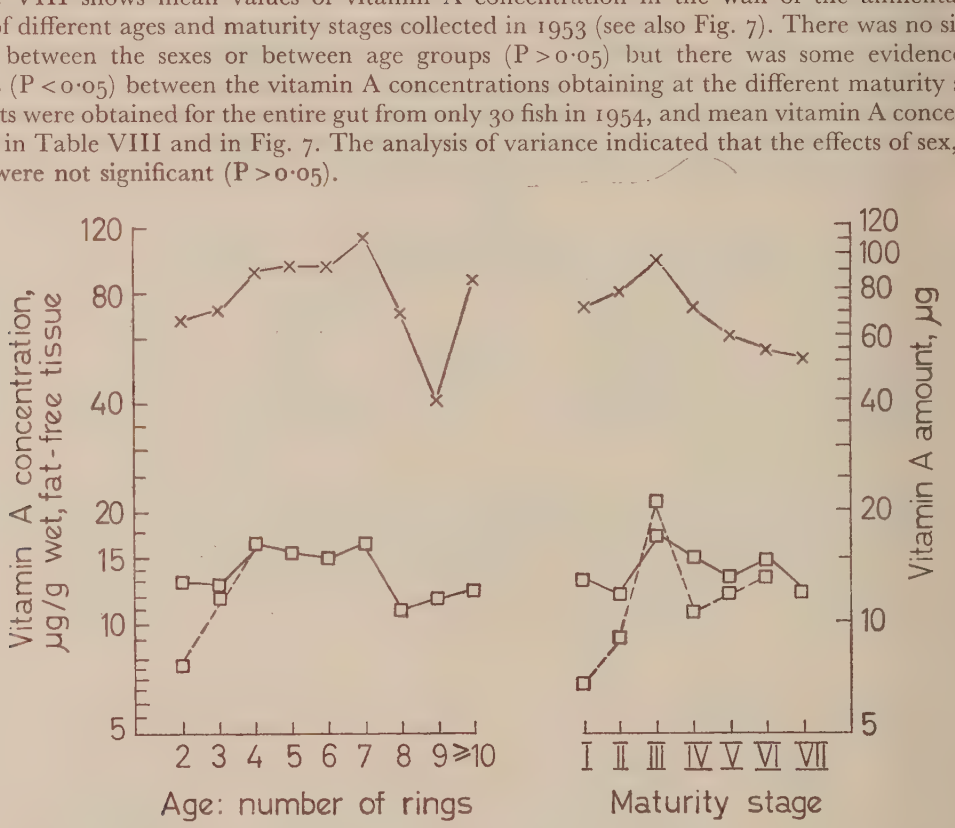

Fig. 7 Vitamin A concentration in the alimentary canal of herring at different ages and maturity stages collected in 1953 □—□ and 1954 □---□, and the amount of vitamin A in the alimentary canal of the 1953 ×—× fish.

Mean values for the absolute amount of vitamin A in the gut wall of the fish taken in 1953 are show in Table VIII and Fig. 7. The amount of the vitamin in male fish did not, on average, differ significantl from that in females, but significant differences due to age ($P < 0{\cdot}05$) and to maturity ($P < 0{\cdot}01$) wer found. Fig. 7 shows that the vitamin A content tended to increase in herring with from 2 to 7 rings an then to decrease erratically. With maturity, increases in the amount of vitamin A from stage I to stag III preceded decreases in the vitamin from III to VII.

In 1953, the fat concentrations in the walls of the alimentary canals from 775 fish of different ag and at different maturity stages were determined (Table VIII, Fig. 8). The analysis of variance showe that the difference in fat content between sexes was insignificant, but the effects of both age and maturit were highly significant ($P < 0{\cdot}01$). Fat concentration decreased progressively with increasing maturit and with increasing age; the proportional decrease with age was virtually constant at 1·13 g fat pe 100 g wet, fat-free tissue per year for all maturities.

Fat concentrations in the gut wall were determined in 1954 in the same 30 fish as were examine for vitamin A, and mean values at different ages and maturities are shown in Table VIII and Fig. 8 There were no significant differences due to age, maturity or sex. Fig. 8 shows in the 1954 results som decline in mean fat concentration with age from 2- to 3-ring fish followed by an appreciable increas

in the 4-ring, but there were observations from only four fish in this age group. Even though the analysis of variance did not indicate any significant differences between fat concentrations at different maturities, Fig. 8 shows, as in the 1953 results, the same trend of decreasing concentration with increasing maturity.

Oesophagus and caecum

Vitamin A was determined in the walls of the oesophagus and caecum of 38 herring sampled in 1954 (Table IX and Fig. 5). The concentration was recorded as nil in 17 fish and was found to exceed 5 μg/g wet, fat-free tissue in four fish, for which the values were 6·7, 12, 14 and 38 μg/g. An analysis of variance

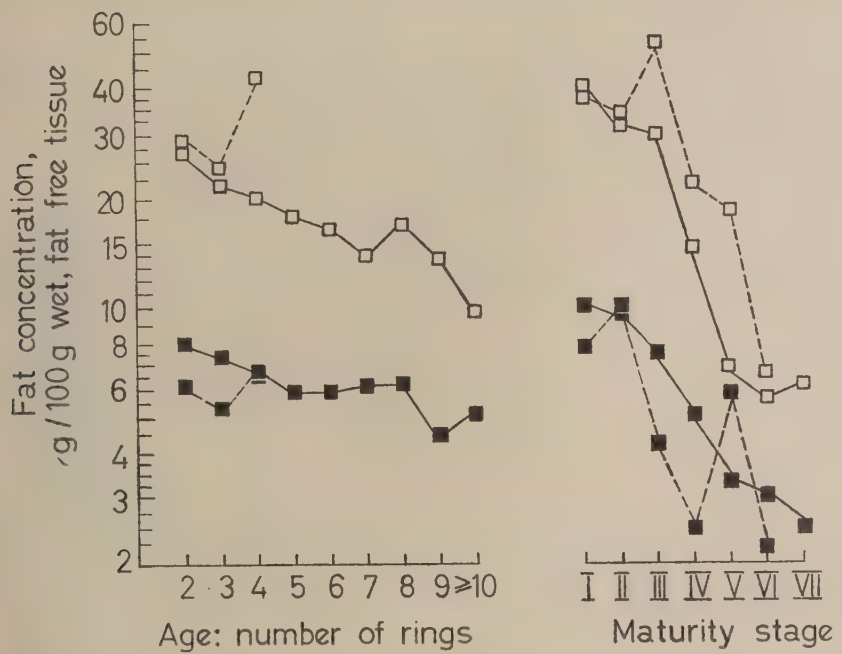

Fig. 8 Fat concentration in the alimentary canal of herring at different ages and maturity stages collected in 1953 □—□ and 1954 □---□ and in the livers of 1953 ■—■ and 1954 ■---■ fish.

on all the results indicated that the effects of age and maturity stage were not significant ($P > 0{\cdot}05$) but that this organ in the male fish was substantially richer in vitamin A than in the female fish ($P < 0{\cdot}01$), the mean values being 2·63 and 0·25 μg/g respectively.

The fat concentration in the oesophagus and caecum of the same herring (Table IX and Fig. 6) ranged from 0·4 to 105 g/100 g wet, fat-free tissue but did not differ significantly with age, maturity or sex.

Pylorus

The mean concentration of vitamin A in the wall of the pylorus of 39 fish taken in 1954 is shown in Table IX and Fig. 5. None was found in the pylorus from 21 herring, and in the remaining 18 fish vitamin A values averaged 7·3 μg/g wet, fat-free tissue and ranged from 2·0 to 150 μg/g, the four values exceeding 10 μg/g being 16, 19, 28 and 150. No values between 0 and 2·0 μg/g were obtained possibly because of the small weights of material analysed.

Fat concentration in the pylorus from the same 39 fish (Table IX and Fig. 6) ranged from 0·3 to 67 g/100 g wet, fat-free tissue, except for values of 93, 119 and 271 g/100 g. No significant differences between fat concentrations in fish of different ages, maturity stages or sexes were obtained.

Pyloric caeca

The mean concentration of vitamin A in the wall of the pyloric caeca from 38 fish sampled in 1954 is shown in Table IX and Fig. 5. The vitamin was present in 36 out of the 38 organs examined and in four of them its concentration exceeded 65 μg/g wet, fat-free tissue, the maximum value being 124 μg/g. Vitamin A concentration did not differ significantly with age, maturity or sex.

The fat concentration in these pyloric caeca (Table IX and Fig. 6) ranged from 0·2 to 170 g/100 g wet, fat-free tissue. The differences in fat concentration with age were significant ($P < 0{\cdot}05$), but not those with maturity and sex. The mean values for 2-, 3- and 4-ring herring were 18, 17 and 65 g/100 g respectively.

Proximal intestine

The mean concentration of vitamin A in the wall of the proximal part of the intestine of 38 fish in 1954 is shown in Table IX and Fig. 5. Vitamin A was found in the proximal intestines from 32 fish, the highest values recorded being 83, 86, 130 and 526 μg/g and the remainder not exceeding 50 μg/g wet, fat-free tissue. The sex, age and maturity differences were not significant ($P > 0{\cdot}05$).

TABLE IX

VITAMIN A AND FAT CONCENTRATIONS IN THE DIFFERENT PARTS OF THE WALL OF THE ALIMENTARY CANAL OF HERRING COLLECTED IN 1954

	Oesophagus and caecum	Pylorus	Pyloric caeca	Proximal intestine	Distal intestine	Mesenteric fat
			Vitamin A concentration (μg/g wet, fat-free tissue)			
Number of fish	38	39	38	38	37	37
Mean concentration	1·19	1·66	13·8	13·5	18·3	3·96
95% confidence limits of mean	0·65, 1·91	0·70, 3·17	8·22, 22·9	7·21, 24·7	10·6, 31·4	2·76, 5·54
			Fat concentration (g/100 g wet, fat-free tissue)			
Number of fish	38	39	38	38	37	37
Mean concentration	11·2	9·83	20·7	35·0	28·0	92·0
95% confidence limits of mean	8·61, 14·5	5·80, 16·2	13·9, 30·6	19·1, 63·3	17·5, 44·5	67·0, 126

Except for an extremely high value of 5163 g/100 g wet, fat-free tissue, fat concentrations in the same 38 proximal intestines (Table IX and Fig. 6) ranged from 0·1 to 395 g/100 g and were apparently unconnected with differences in sex, age or maturity.

Distal intestine

The mean vitamin A concentration in the wall of the distal part of the intestine from 37 fish sampled in 1954 is shown in Table IX and Fig. 5. Vitamin A was present in 32 of the distal intestines examined. The highest values recorded were 143, 148, 186 and 361 μg/g wet, fat-free tissue, but among the remainder no concentration exceeded 78 μg/g. Sex, age and maturity differences were not significant ($P > 0{\cdot}05$).

Fat concentrations in the distal intestines of the same 37 fish (Table IX and Fig. 6) included values between 1·1 and 245 g/100 g wet, fat-free tissue and others of 348, 726 and 1941 g/100 g. There were no significant differences in fat concentration with sex, age or maturity.

Mesenteric fat

The fat attached to the intestinal wall of 37 fish taken in 1954 was analysed for vitamin A (Table IX). The vitamin was not found in the mesenteric fat of three fish, and occurred in concentrations of less than 10 μg/g wet, fat-free tissue in 26 and greater than 25 μg/g in three fish, the highest value being 58 μg/g. The differences in vitamin A concentration with sex and age were not significant ($P > 0{\cdot}05$) but those of maturity were ($P < 0{\cdot}05$); the mean concentrations in the fish taken at maturity stages I to VI were 6·8, 2·8, 10·9, 1·8, 12·0 and 0·0 μg/g respectively.

Apart from higher values of 310, 485, 527, 1126 and 2010 g/100 g wet, fat-free tissue in five fish, l the recorded fat concentrations in the mesenteric fat were less than 240 g/100 g. The male fish were gnificantly richer in fat than the females ($P < 0.05$) and the respective mean values were 140 and 56 100 g. The maturity effects on fat concentration were also significant; the mean values for fish in stages to VI were 760, 81, 103, 60, 104 and 7 g/100 g respectively, showing that the immature fish had by far e highest concentrations of fat in this layer.

VER

The distribution of vitamin A concentration in the livers of 777 herring collected in 1953 is shown Fig. 9 in terms of μg/g wet weight of fat-free tissue and in Fig. 10 in terms of the logarithmically ansformed values.

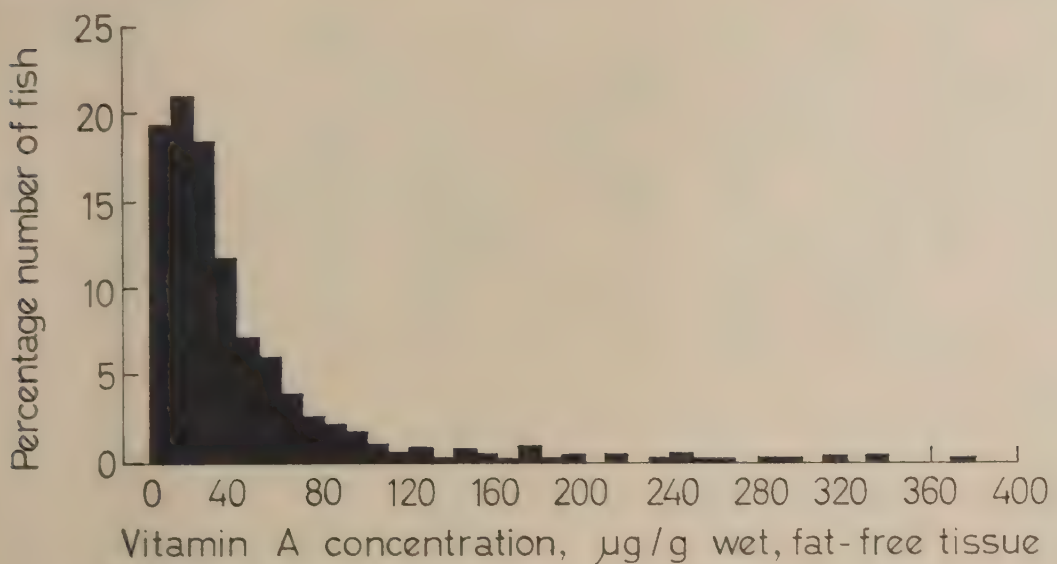

Fig. 9 The distribution of vitamin A concentrations in the livers of 777 herring collected in 1953.

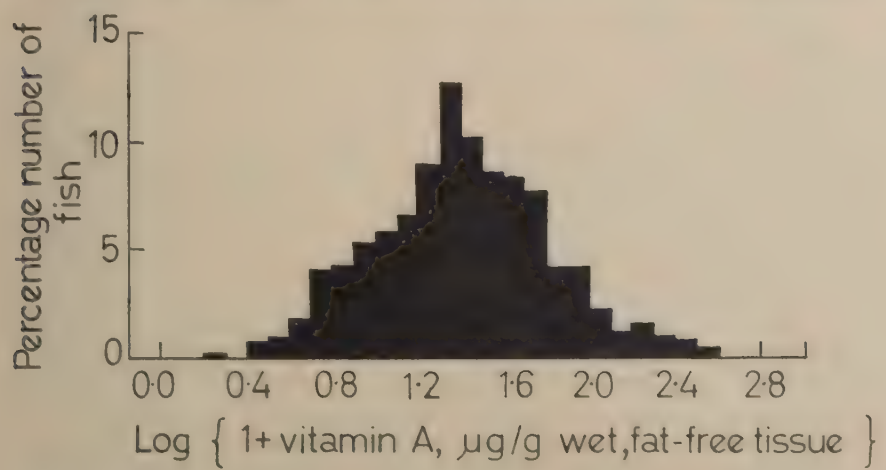

Fig. 10 The distribution of the logarithmically transformed vitamin A concentrations in the livers of 777 herring collected in 1953.

The first analysis of variance having shown the residual mean square after fitting age, maturity an sex to be significant ($P < 0{\cdot}01$), the transformed vitamin A concentrations for 385 male and 392 fema herring were analysed separately. Mean values are given in Table X. The differences in vitamin concentration with age were found to be highly significant for both sexes ($P < 0{\cdot}01$) and those wit maturity were significant in male ($P < 0{\cdot}01$) but not in female fish ($P > 0{\cdot}05$). The general trend wit age (Fig. 11) was for the concentrations in both male and female to increase proportionately at a approximately constant rate from 2 to 6 rings and to decline in the older (>8-ring) fish. In relation t

TABLE X

MEAN VALUES FOR VITAMIN A AND FAT IN THE LIVER OF HERRING COLLECTED IN 1953 AND 1954. VITAMIN A VALUES FOR MALE (♂) AND FEMALE (♀) FISH TAKEN IN 1953 ARE GIVEN SEPARATELY

Ring-age	Vitamin A concentration (μg/g wet, fat-free tissue)			Vitamin A amount (μg)		Fat concentration (g/100 g wet, fat-free tissue)	
	1953		1954	1953		1953	1954
	♂	♀	♂+♀	♂	♀	♂+♀	♂+♀
2	17·7	16·7	7·25	28·6	39·4	7·90	6·14
3	24·0	22·7	13·0	48·3	66·5	7·25	5·29
4	34·4	41·7	27·7	82·0	121	6·69	6·70
5	42·4	46·7	—	78·5	164	5·84	—
6	61·7	55·5	—	135	190	5·84	—
7	28·3	66·1	—	87·3	245	6·05	—
8	56·3	68·4	—	147	255	6·12	—
9	26·2	40·4	—	78·2	158	4·46	—
⩾ 10	29·1	38·3	—	76·4	150	5·06	—
Maturity stage							
I	22·1	27·2	7·30	47·7	60·0	11·0	7·76
II	16·8	25·1	10·3	38·7	65·6	9·58	10·1
III	30·9	22·9	14·3	60·6	81·9	7·43	4·21
IV	42·1	25·5	14·3	67·1	89·8	5·01	2·47
V	31·5	22·7	11·7	37·6	80·7	3·32	5·79
VI	28·6	42·2	10·6	43·3	85·0	3·01	2·20
VII	20·3	15·3	—	34·8	40·6	2·47	—
Total fish	385	392	39	385	392	791	40
Mean value	24·4	24·3	11·2	46·2	66·7	7·12	5·81
95% confidence limits of mean	22·3, 26·7	22·4, 26·4	8·70, 14·3	42·4, 50·5	61·7, 72·2	6·85, 7·41	4·77, 7·04

maturity (Fig. 11) vitamin A concentration in the liver of male herring increased during the early stage of maturation and decreased from stage IV onwards whereas, in the female, concentrations were obviously similar up to stage V with some increase in the ripe fish and a decline at spawning.

Mean vitamin A concentrations in the livers of 39 fish collected in 1954 are given in Table X. I contrast with the 1953 results, the residual mean square after fitting age, maturity and sex was no significant ($P > 0{\cdot}05$) and the results for male and female fish were not treated separately. The analysi of variance showed significant age differences but no real difference in vitamin A concentration betwee sexes or maturities. As far as could be judged from the few results of 1954, age and maturity trends in th vitamin A concentration in the liver of male and female fish were like those already obtained in the 195 results.

In addition to the concentrations, the absolute amounts of vitamin A in the liver of herring taken i 1953 were studied (Table X). The results from males and females were examined separately and analyse of variance showed that the amount of vitamin A in the liver was significantly affected by age ($P < 0{\cdot}01$ and by maturity ($P < 0{\cdot}05$) in both sexes. Mean values given in Table X show that the amount of vitami

A was consistently greater in the female than in the male herring of like age and maturity and the age and maturity trends for males and females are shown separately in Fig. 11. The proportional increase in vitamin A was highest, and similar for both sexes, at from 2 to 4 rings, after which changes tended to become progressively smaller in the female, and to be very small in the male. The amount of vitamin A in the liver of both male and female fish increased up to maturity IV but after this stage there was a general decline in the male whereas in the female the vitamin remained at about the same level until stage VI and then decreased rapidly at spawning (Fig. 11).

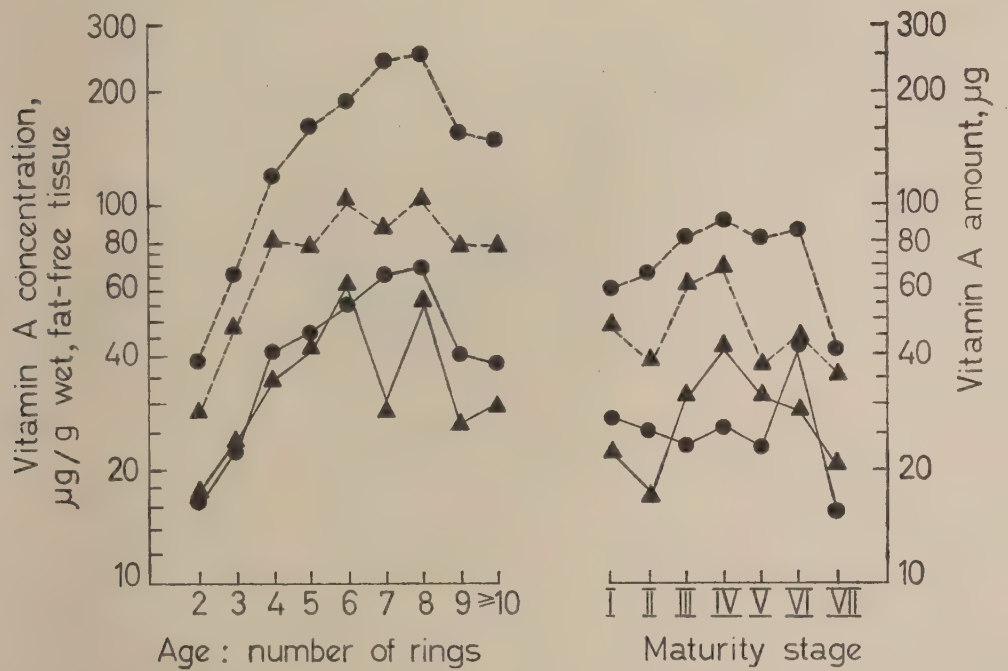

Fig. 11 The amount and concentration of vitamin A in the liver of male ▲---▲, ▲—▲ and female ●---●, ●—● herring at different ages and maturities taken in 1953.

The livers of 791 herring collected in 1953 were analysed for fat content (Table X, Fig. 8). The fat concentrations in the livers of male and female herring were similar, but differed significantly with both age and maturity grading ($P < 0{\cdot}01$). Proportional decreases in liver fat concentration tended to be constant at 1·06 g fat per 100 g wet, fat-free tissue per year for all maturities and the progressive decrease with advancing maturity was similar at all ages.

Fat concentration was also determined in the livers of the 40 fish taken in 1954 (Table X, Fig. 8). The analysis of variance indicated that differences attributable to stage of maturity were significant ($P < 0{\cdot}01$) but those attributable to age and sex were not ($P > 0{\cdot}05$). The relationships of fat concentration with age and maturity (Fig. 8), though less well-defined than those obtained with the more numerous results of 1953, generally agreed with them.

The wet weight of the fat-free tissue in the 777 livers examined for vitamin A was also studied. The weights of fat-free liver tissue in males and females were analysed separately; the mean value with 95% confidence limits was $2{\cdot}14 \pm 0{\cdot}08$ g in 385 male and $3{\cdot}06 \pm 0{\cdot}09$ g in 392 female herring. Differences in weight with age and maturity were highly significant ($P < 0{\cdot}01$) in both sexes. In the male the changes occurring with maturation were similar at all ages whereas in the female maturity trends were not independent of the age of the fish. The decreases in tissue weight with maturity up to stage V and the increases at spawning were similar in males of different ages, but in females the weight of fat-free liver

tissue increased during early maturity and decreased in mature and spawning fish, larger changes occurring with increasing age (Fig. 12). The pooling of different age groups, although not entirely appropriate, permitted these limited comparisons to be made even though there were very few or no female herring in some of the age-maturity groups.

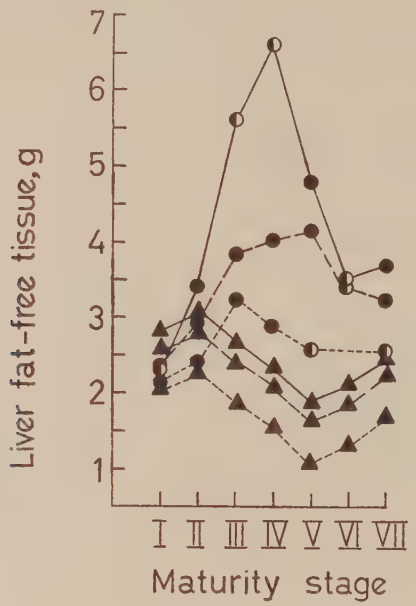

Fig. 12 The weight of fat-free tissue in the liver at different maturities for 2-ring ●····●, 3-4-ring ●---● and 5-8-ring ●—● female fish and for male fish ▲ of corresponding ages. Mean values for female fish based on more than 5 observations are shown ● and on 5 or fewer results ◐.

Liver colour was noted in 282 fish taken in 1953. Table XI shows the mean values and the results of significance tests for the fat concentration in the liver and the muscle of herring with livers of the same colour.

TABLE XI

MEAN VALUES FOR FAT CONCENTRATION (g/100 g WET, FAT-FREE TISSUE) IN THE LIVER AND MUSCLE OF HERRING WITH LIVERS OF DIFFERENT COLOUR

	Liver colour			
	Dark red	Red	Yellow	Mottled
Number of herring	52	145	44	41
	Fat concentration in the liver			
Mean*	2·60	7·07	10·9	11·3
	Fat concentration in the muscle			
Mean*	12·0	12·6	14·2	16·4

*Any two mean values underlined by the same line are not significantly different ($P>0.05$) and, conversely, any two means not underlined by the same line are significantly different ($P<0.05$).

ONADS

The gonads of 757 herring collected in 1953 were examined for vitamin A, which was found in easurable quantities in 110 out of 367 testes and in 201 out of 390 ovaries. The frequency with which was present in the female was significantly different from that in the male ($\chi^2=36{\cdot}3$, $P<0{\cdot}01$). In lay and June when most of the fish taken were of maturities I and II, vitamin A was found in 21% nd 34% of testes and ovaries respectively, but in July and August the corresponding values were 37% nd 68% for herring generally more mature. The percentages of herring with vitamin A in the gonads the different maturity stages are shown in Table XII: vitamin A was present more often at stages III, V and V than at others, and more frequently in the females than in the males at the same maturity stage.

Table XII also shows the mean concentrations of vitamin A in the gonads of fish at different maturities nd with the vitamin present in measurable amounts. The concentration of vitamin A in gonads was enerally found to be less than 1 μg/g wet, fat-free tissue: the values exceeding 5 μg/g were 7·3, 8·9 and 0·0 in milt and 6·0 in roe. Even though it was found less often in the male, vitamin A, when present, ould occur in similar concentrations in the testes and ovaries.

The mean concentration of vitamin A in the gonads from all the 757 herring was 0·17 μg/g wet, t-free tissue with 95% confidence limits of 0·14 and 0·19 μg/g. The differences in vitamin concentration ue to maturity and to sex were highly significant ($P<0{\cdot}01$). The concentration was highest in the nmature fish, declined with increasing maturity up to stage VI and increased to the final stage at which itamin concentration in the spent fish approached that in stage II herring (Fig. 13). Vitamin A in stis was less than in ovary by approximately 0·10 μg/g wet, fat-free tissue.

TABLE XII

VITAMIN A IN THE GONADS OF MALE (♂) AND FEMALE (♀) HERRING TAKEN IN 1953

Maturity stage	Number of herring		Number with vitamin A in gonads		Percent with vitamin A in gonads		Mean concentration of vitamin A when present in gonads (μg/g wet, fat-free tissue)	
	♂	♀	♂	♀	♂	♀	♂	♀
I	81	96	23	36	28	38	1·31	1·27
II	118	134	21	54	18	40	0·74	0·74
III	34	56	15	36	44	64	0·15	0·24
IV	52	34	24	28	46	82	0·07	0·11
V	32	28	16	24	50	86	0·05	0·10
VI	36	7	8	5	22	71	0·02	0·06
VII	14	35	3	18	21	51	0·55	0·41
All stages	367	390	110	201	30	52	0·40	0·48

The absolute amount of the vitamin in the same gonads averaged 0·52 μg with 95% confidence mits of 0·46 and 0·57 μg. Differences in the amount of the vitamin with maturity and sex were very rge but maturity trends were not the same for male and female herring. Fig. 13 shows the mean values r males and females separately at each maturity stage and it will be seen that the testis-ovary difference as much larger at stages IV, V and VI than at the other maturities, the increase in the amount of tamin A in the ovary during maturation being markedly greater than the increase in the testis.

The mean value of fat concentration in the gonads of 757 fish sampled in 1953 was 3·02 g/100 g et, fat-free tissue with 95% confidence limits of 2·85 and 3·20 g/100 g (Fig. 14). Analysis of variance dicated no significant differences between the fat concentrations of testis and ovary or between those the gonads of fish of different ages, but the differences in concentration were highly significant ($P<0{\cdot}01$) respect of maturity, fat concentration decreasing with increasing maturity (Fig. 14).

The absolute weight of fat in the same gonads was also determined and the mean was 113 mg with 5% confidence limits of 106 and 119 mg. As with fat concentration, the testis-ovary difference was ot significant ($P>0{\cdot}05$) and the differences with maturity were highly significant ($P<0{\cdot}01$). The mean

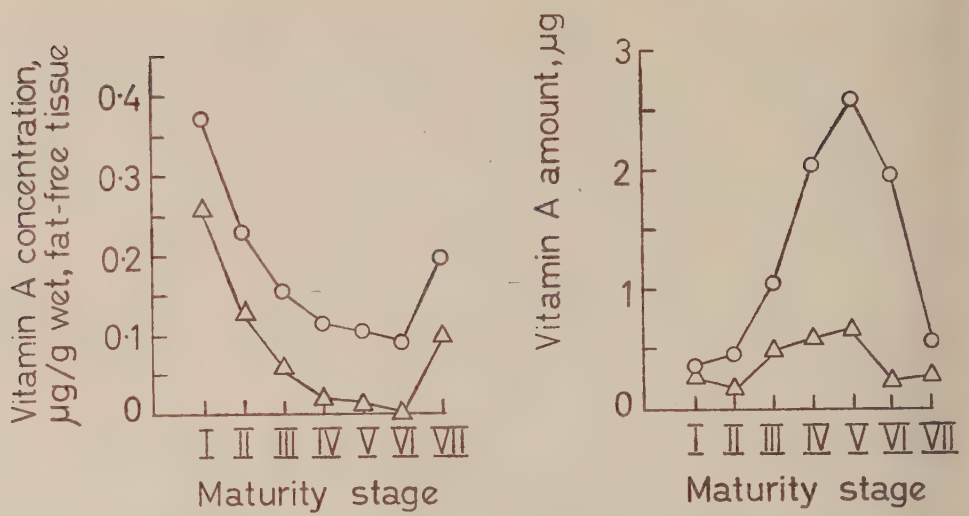

Fig. 13 The concentration and the amount of vitamin A in the gonads of male △—△ and female ○—○ fish according to the maturity stage when taken in 1953.

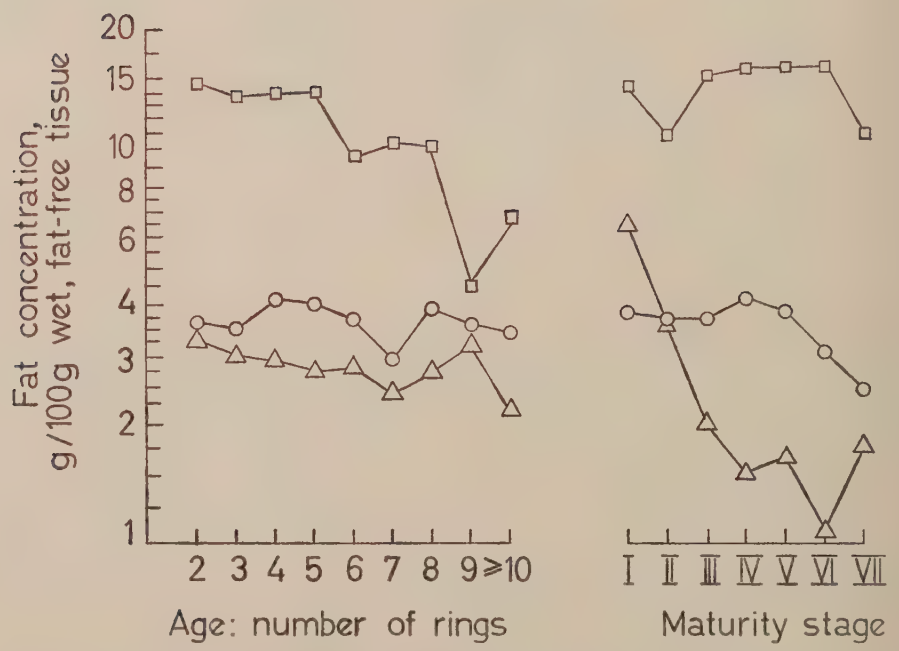

Fig. 14 Fat concentration in the gonads △—△, the eyes ○—○ and the muscle □—□ of herring at different ages and maturity stages collected in 1953.

eight of fat in the testes was 109 mg and in the ovaries 116 mg. Mean values for fish at stages I to VII re 54, 67, 201, 408, 542, 388 and 42 mg respectively.

ES

The eyes of 781 herring collected in 1953 were examined for vitamin A content. The average ncentration was 1·15 μg/g wet, fat-free tissue with 95% confidence limits of 1·10 and 1·20 μg/g. fferences with sex and age were small, but differences with maturity were much larger and statistically nificant ($P < 0{\cdot}05$). The mean values determined for maturity stages I to VII were 1·14, 1·27, 1·07, 7, 1·15, 0·94 and 0·98 μg/g respectively.

Fat was determined in the eyes of 791 fish taken in 1953 and the mean concentration was 3·64 00 g wet, fat-free tissue with 95% confidence limits of 3·49 and 3·80 g/100 g. There was no difference eye fat concentration between fish of different sexes or ages ($P > 0{\cdot}05$). Maturity effects were, however, nificant ($P < 0{\cdot}01$); the fat concentration changed only slowly during maturation but declined rapidly the ripe and spent fish (Fig. 14).

USCLE

The determination of the fat in the body muscle of 289 fish collected in 1953 was carried out at berdeen. The mean concentration was 13·2 g/100 g wet, fat-free tissue and the 95% confidence limits re 12·5 and 14·0 g/100 g. Differences in fat concentration with sex were not significant but those with e and maturity were highly significant ($P < 0{\cdot}01$). Fig. 14 shows that muscle fat concentration was ghest in herring of 2 to 5 rings and appreciably lower in the older fish: the concentrations in stage II d spent herring were similar and much lower than in immature, maturing or ripe herring.

Group Herring

Vitamin A concentration was determined in the pyloric and intestinal part of the alimentary canal 19 and in the oesophagus and its caecum and the liver of twenty 1-group fish collected in December 54. Mean values are given in Table XIII. The mean vitamin A concentration in the male did not ffer significantly from that in the female fish in any of the organs examined. The concentration in the sophagus and caecum was about five times greater than in the rest of the alimentary canal and only ghtly less than that in the liver.

Values for fat were also obtained from these 1-group herring (Table XIII). Fat concentration in e male differed significantly from that in the female only in the pyloric and intestinal part of the mentary canal ($P < 0{\cdot}05$). The average concentration of fat in the entire gut of the 1-group fish was tween two and three times greater than in the liver.

TABLE XIII

MEAN VALUES FOR VITAMIN A AND FAT CONCENTRATION IN THE ALIMENTARY CANAL AND THE LIVER OF 10 MALE AND 10 FEMALE 1-GROUP HERRING TAKEN IN 1954

	Oesophagus and caecum	Pyloric and intestinal gut	Entire gut	Liver
	Vitamin A concentration (μg/g wet, fat-free tissue)			
ale fish	21·6	4·91	11·5	21·5
male fish	18·6	2·93*	8·35*	24·2
l fish	20·1	3·87‡	9·88‡	22·8
% confidence limits of mean	14·1, 28·4	2·68, 5·45	7·27, 13·3	14·4, 35·7
	Fat concentration (g/100 g wet, fat-free tissue)			
ale fish	38·0	55·1	44·7	12·0
male fish	33·2	24·2*	27·6*	15·1
l fish	35·5	37·4‡	35·6‡	13·5
% confidence limits of mean	25·6, 49·2	26·3, 53·0	26·3, 48·0	9·32, 19·2

*9 herring only. ‡19 herring only.

0-Group Herring

Vitamin A was determined in forty-two 0-group herring taken in December 1954 (Table XIV). The concentration (amount of vitamin A per unit wet, fat-free tissue of the whole fish) was apparent unrelated to body-weight. The amount of vitamin A in the young fish, however, increased with increasi total body-weight, the proportional increase tending to be constant at 1·09 μg/g body-weight, wi 95% confidence limits of 1·01 and 1·18 μg/g.

TABLE XIV

MEAN VALUES FOR VITAMIN A AND FAT IN FORTY-TWO 0-GROUP HERRING COLLECTED IN 1954 AND GROUPED ACCORDING TO TOTAL BODY WEIGHT

Weight of fish			Vitamin A		Fat	
Range (g)	Mean (g)	Number of fish	Concentration (μg/g wet, fat-free tissue)	Amount (μg)	Concentration (g/100 g wet, fat-free tissue)	Amou (g)
2·6- 6·0	4·2	24	0·49	1·87	6·25	0·24
6·1- 9·5	8·1	9	0·39	2·92	5·61	0·42
9·6-13·0	10·8	9	0·39	3·86	7·09	0·69
2·6-13·0	6·5	42	0·45	2·40	6·28	0·34

The weight of fat was also recorded for these 0-group herring (Table XIV). Fat concentrati (weight of fat per 100 g wet, fat-free tissue in the carcase) showed no systematic change with changi body-weight. The proportional increase in the total amount of fat in the whole fish was virtually consta at 1·16 g fat/g increase in body-weight, 95% confidence limits being 1·08 and 1·25 g/g.

DISCUSSION

Vitamin A

The brief summary of previous work on vitamin A in the herring given in the introduction to th paper indicates that this study, although far from complete, is probably the most extensive yet mad Even so, many problems require further investigation.

In marked contrast to the finding of vitamin A in the majority of the 1954 plankton samples, no was detected in those of 1953. This cannot be accounted for by the smaller amounts of material obtain in the first year because no Carr-Price reaction occurred even with the bulked extract from all the 19 samples. The difference between the observations for the two years is likely to have been greatly affect by the composition of the samples resulting from the method of hauling: previous and subseque experience has shown that a much higher proportion of actively swimming animals, such as euphausi crustaceans, would be caught in oblique hauls, as used in 1954, than in vertical hauls, as used in 195 and superficial examination of the samples indicated that this was so. Moreover, there was great likelihood of catching euphausiids in 1954 because, according to Glover (1957), this was the mo productive year from 1952 to 1955 for euphausiids in the North Sea off north-east Scotland. That plankt of composition similar to that which the herring were eating was not being caught in 1953 is suggest by the results in Table V which show that vitamin A was found in 28% of the caecal contents from t herring taken in that year.

Fig. 4 shows that the organisms forming the food of these herring were similar to those reported l earlier workers, e.g. Hardy (1924), Ogilvie (1934), Savage (1931, 1937) and Jespersen (1944). All accour emphasise the importance of copepods in the diet, with Larvacea, gastropods, euphausiid and decap crustaceans, chaetognaths, and young fish, especially *Ammodytes*, as other components of variable val according to season, locality and other environmental factors.

Vitamin A and carotenoids have been determined in species from most of these groups. In copepo vitamin A and its precursor β-carotene occur only rarely (Fisher & Kon, 1959; Fisher & Goldie, 195 the predominant carotenoid being astaxanthin. Copepods can thus contribute little directly to t

amin A reserves of herring feeding on them, but the occurrence in them of a yet unidentified precursor nnot be neglected (Fisher & Goldie, 1959) and the possibility that astaxanthin may be converted to amin A must also be considered. Grangaud & Massonet (1955) claimed that the poeciliid fish *Gambusia brooki* can convert astaxanthin to vitamin A and unpublished work at Shinfield on this species gave a nilar indication. In an experiment with much more carefully purified astaxanthin, however, *Lebistes culatus*, another poeciliid, did not effect this conversion although it did accumulate the vitamin when on a diet containing β-carotene.

Hardy (1924) found that *Ammodytes* could be an important part of the herring's food, but it did not cur very frequently in the fish investigated (in Fig. 4 it would be included under "young fish"). Con-ntrations of vitamin A in livers of *A. lanceolatus* analysed subsequently (Fisher, 1955) were low and rotenoids occurred only occasionally.

The gastropods eaten by the herring are pteropods which may make a direct contribution to the amin A reserves. *Spiratella retroversa* (Fisher *et al.* 1956*b*) and *Euclio cuspidata* are quite rich in the vitamin t none has been found in *Clione limacina* and *Euclio pyramidata* (Fisher & Kon, 1959). *Clione limacina* es contain measurable amounts of β-carotene (Fisher *et al.* 1956*b*) but pteropods generally are poor carotenoids.

The chaetognaths most likely to be available to herring in the North Sea are species of *Sagitta* and ese contain no vitamin A (Fisher & Kon, 1959), only small concentrations of carotenoids and no carotene. Nothing is known of the vitamin A and carotenoid contents of Larvacea.

Euphausiids, probably the most important higher crustaceans in the food of the herring, are rich vitamin A (Fisher *et al.* 1955) and their major, if not only, carotenoid is astaxanthin. Other marine agic Crustacea that herring may eat contain little or no vitamin A (Fisher & Kon, 1959) and, at ost, only small amounts of carotenoids (Goodwin, 1960).

The value of those organisms making a direct contribution to the vitamin A reserves of the herring viously depends upon their seasonal abundance and annual variation. Thus Gillam *et al.* (1939) found at bulk plankton samples from the North Sea were far richer in vitamin A and carotenoids in May an during the other months of the year. Table III agrees with their finding, in that the concentrations vitamin A in plankton collected in April 1954 and from the northern half of the area in May 1954 re higher than in later months. Glover (1956) included euphausiids among the organisms characteristic the richer "oceanic" water found in the northern area in May 1954. Gillam *et al.* (1939) recorded much gher numbers of organisms but lower vitamin A and carotenoid concentrations in their August than their May plankton samples. Only 63% of the animals in May were copepods, whereas in August ese formed 85% of the total; euphausiids or other vitamin A-containing forms may well have made this difference in May. In the August samples (Table III) concentrations of carotenoids were relatively gh in both years indicating the presence of Crustacea rich in astaxanthin and poor in vitamin A, that copepods rather than euphausiids.

An attempt to relate the incidence of vitamin A in the plankton samples to the animals present in em is made in Table IV but each sample contained several animal groups which were not separated fore analysis. Thus all the samples containing coelenterates and ctenophores had vitamin A present, t the vitamin has not been found in these groups (Fisher & Kon, 1959) and its presence in the samples s probably due to euphausiids or young fish. There is no doubt that the herring obtains part of its amin A preformed in its food and that the proportion depends on the quantity of euphausiids and ner vitamin A-containing organisms eaten. So far not enough is known about the metabolism by fish possible precursors of vitamin A in, for example, copepods to determine whether the requirements of e herring are satisfied by the amount of preformed vitamin in different kinds of plankton.

The vitamin A content of young herring increases with their size (Table XIV). Earlier investigators ebour, 1923-5; Marshall *et al.* 1938; Hentschel, 1950) have shown that herring larvae feed first on ytoplankton and later on copepods. The phytoplankton, mostly diatoms, would provide β-carotene nich may be converted to vitamin A. After metamorphosis of the herring larvae, copepods form the lk of the food (Marshall *et al.* 1939), although other organisms, probably including forms containing amin A, are taken when abundant in the plankton.

Only about a third of the food samples from oesophageal caeca contained vitamin A (Tables V and

VI). As the fish became sexually mature towards the end of July many of them stopped feeding and caecal contents in these fish, being in a compact mass of unidentifiable material rather than a mixture easily separated animal fragments, often appeared to have been in the gut for some time. Exceptiona high concentrations of vitamin A occasionally occurred in these contents which were rich in fat but v poor in carotenoids.

The series of gut contents examined showed that vitamin A could occur in similar concentrati in all parts of the alimentary canal; but the vitamin was found rarely in the contents of the pylor only in the contents of the intestines of about one in five, and only in the pyloric caeca of two in fi fish. The mean values for all contents from one part of the gut (Fig. 5) may therefore indicate only relative importance of that part as a site of occurrence of vitamin A in the contents of the alimenta canal.

Vitamin A was more concentrated in the walls of the pyloric caeca and the intestine, regarded Barrington (1957) as the absorptive parts of the alimentary canal, particularly for fatty substances, th in those of the oesophagus and its caecum and the pylorus (Table IX). The herring differed in t respect from the rat in which the concentrations of vitamin A were highest midway along the sm intestine and declined in the distal half (Thompson *et al.* 1950). In the sperm-whale as in the herri however, Wagner (1939) found that vitamin A concentrations were as high in the hind gut as in the m gut. The low concentrations in the wall of the oesophagus and pylorus and in the mesenteric fat indic that these tissues are relatively unimportant in the adult herring as storage sites for vitamin A. In t 1-group fish the concentration of vitamin A in the oesophagus and its caecum was much higher than the remainder of the gut (Table XIII), but in the adult herring in 1954 the vitamin concentration v lower in the oesophageal caecum (wall and contents) than in the pyloric and intestinal parts (Fig. 5).

Increase in the liver reserves of vitamin A with age was probably first observed in the Newfoundla cod (Macpherson, 1933: Macpherson & Wilson, 1935). Apart from general statements that such accumulation takes place in fish (Moore, 1957, p. 591), no more precise information on the herring other species has been found. In the herring vitamin A accumulated in the liver, as in the alimenta canal, with increasing age up to 8 rings and tended to fall off again in older fish (Table X, Fig. 11).

The amounts of vitamin A in the alimentary canals of male and female herring follow closely simi trends in relation to maturity, increasing up to stage III and then decreasing steadily in the later sta (Fig. 15). Increases in the liver vitamin A reserves in both sexes also occur during the early matur stages but do not continue after stage IV, and cannot ultimately account for the losses from the gut wa However, whereas in the male there is a sharp drop in liver reserves of vitamin A after stage IV, in t female the reserves are maintained at a high level until spawning actually occurs.

The vitamin A concentration and content of both male and female gonads were never very hi (Fig. 13), in marked contrast to the results of earlier work in which biological assays were used (Scheun & Schieblich, 1934; Junker, 1952-3). A considerable difference between the vitamin A activity measu biologically and the amounts of vitamin A ester and alcohol determined by the physico-chemical meth has been shown by Fisher *et al.* (1956*a*). Plack *et al.* (1959) have shown this to be due to the presence vitamin A aldehyde which may occur in concentrations ten times greater than that of other forms of t vitamin in the ripe roes of herring and is destroyed by saponification during the chemical analys Junker (1952-3) gave values in terms of whole tissue including fat, and graphs relating vitamin A co centration to maturity appear to be the reverse of those in Fig. 13. But if Junker's results are express as amounts of vitamin A for her maturity grades 2 to 8, probably coinciding with Hjort's maturity grou I-VII, they are respectively 1·5, 5·2, 14·1, 83, 236, 172, and 4·0 μg and, except for much greater valu in the ripe gonads, the maturity trends for roes are then similar to those in Fig. 13.

The testes contain much smaller concentrations of vitamin A but these follow the same trends as the ovaries in relation to maturity (Fig. 13). Junker's (1952-3) values expressed as amounts of vitamin in testes from herring in grades 2 to 6 are 1·2, 4·1, 1·7, 25·2 and 56 μg respectively, showing a simil maturity trend to that in Fig. 13 even though Junker's values for the absolute amounts of the vitam were consistently higher. Preliminary biological tests on extracts from herring testes, however, reveal no evidence of vitamin A aldehyde (Plack, 1960). The differences between the amounts of vitamin in testis and ovary increase as the fish mature and reach maximal values in stages V and VI (Fig. 1

those obtained with Junker's more restricted results. This difference between sexes during the later urity stages may indicate a difference in the metabolism of vitamin A, or it may reflect differences in elopment of the gametes in that the eggs are all formed at one time and transfer of vitamin A probably urs when the yolk is formed (Plack *et al.* 1961) whereas in sperm production new gametes continue e formed until the sperm sac is full.

In the liver, as in the gonads, the biggest differences between males and females in the amounts of min A occurred at stages V and VI (Table X, Fig. 15). The liver of the female retained its vitamin A

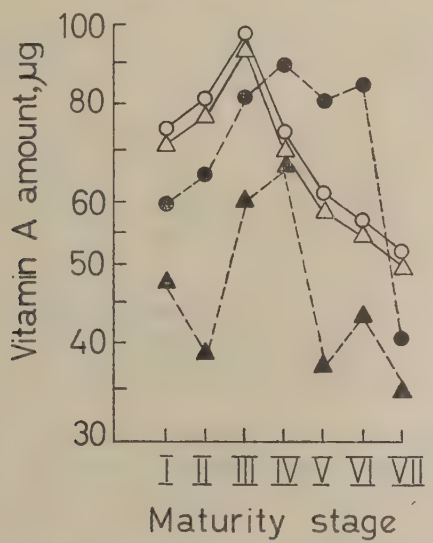

Fig. 15 The amount of vitamin A in the liver and in the intestine of male ▲--▲, △—△ and female ●--●, ○—○ herring at different maturity stages collected in 1953.

that of the male did not at a time when the difference between the vitamin contents of the ovaries testes was greatest. There was, however, no corresponding difference between sexes in the pattern itamin A loss from the alimentary canals.

The drain of vitamin A reserves from the alimentary canal and the liver before or during spawning their loss from the gonads during spawning indicate a possible function in reproduction. Plack 50) has shown that the aldehyde is the predominant form of vitamin A in the ripe ovaries, but little min A was detected in the very small samples of newly hatched larvae so far available for analysis.

Values for vitamin A in the eyes (p. 25) were somewhat lower than those obtained by Edisbury '. (1938) but, like theirs, do not include the aldehyde which is undoubtedly the most abundant form he vitamin in these organs, being part of the visual pigment rhodopsin. However, even with the ll amount of vitamin A ester and alcohol present, there is a fall in concentration at spawning, indicating the fish draw on their reserves, however small, at this critical period in their annual cycle.

Edisbury *et al.* (1938), Lunde (1938), Bacharach *et al.* (1942) and Scheunart *et al.* (1956) using sico-chemical methods found little or no vitamin A in herring muscle and preliminary experiments Shinfield gave similar indication. However, Junker's (1956) biological assays showed vitamin A centrations of 0·15 to 1·26 µg/g in muscle. The concentrations given by different workers appear to related to the analytical method. When a refined assay technique has been developed, the importance he muscle in the vitamin A metabolism of the herring may well have to be reconsidered. With the tively large weight of muscle in the fish, a small concentration could result from a total amount of min A in the muscle tissue of the same order as that in the liver or the alimentary canal.

The results, which do not include vitamin A in muscle, have shown that the alimentary canal the liver and to a much smaller extent the gonads and the eyes contain the vitamin. The amounts pres however, may differ greatly with the age, maturity and sex of the fish.

The relative importance of the main sites of occurrence of vitamin A is difficult to determine as Table XV shows, neither the liver nor the alimentary canal is consistently the richer for both s at all maturity stages.

TABLE XV

THE ABSOLUTE AMOUNT OF VITAMIN A IN THE LIVER EXPRESSED AS A PERCENTAGE OF THAT IN THE ALIMENTARY CANAL OF HERRING TAKEN IN 1953

	Maturity stage						
	I	II	III	IV	V	VI	VII
Male fish	67	50	65	95	64	80	70
Female fish	80	80	83	121	131	149	77

Fat

The plankton samples taken in 1954 were richer in fat than those of 1953, whether we compare values for all the samples or only those for samples taken from the same area in the two years; difference, although less marked than that in vitamin A, probably also depended upon variation in composition of the samples.

The mean fat concentrations in the plankton samples were much lower (14·8 and 22·2 g/100 g fat-free tissue in 1953 and 1954 respectively) than those in the food represented by the contents of oesophageal caeca (50·3 and 52·9 g/100 g). This may have been due to the presence in the plank samples of organisms with low fat content, which by selective feeding the fish may reject. Howe the highest values of fat in the food of the 1953 herring were obtained from fish in stages I and II, most voracious feeders, which were usually caught in the first half of the season (Fig. 2) when plank was abundant and rich in fat (Gillam *et al.* 1939).

The apparently higher fat concentrations in the contents of oesophageal caeca in maturity sta V and VI cannot be correlated with the increased plankton fat values in August because these mat fish were in general not feeding. The fat values, therefore, refer to contents which had probably persis in the caeca for some considerable time after the fish had last fed and from which absorption of fat i the gut was slow relative to that of the other constituents in the food.

The concentrations of vitamin A in the walls of the pyloric and intestinal parts of the aliment canal were much higher than in the respective contents (Fig. 5) even though values in the latter w based on dry weight and in the former on wet weight. On the other hand, the fat concentrations in contents appear greatly to exceed those in the walls (Fig. 6), but when all values are expressed in te of wet weight, on the assumption that contents, like plankton, contain about 70% water (Fisher *et* 1952), they are closely comparable, except in the pylorus. However, as with vitamin A, the absorpt parts of the canal had higher fat concentrations in the wall than the oesophagus and pylorus (Table I Fig. 6).

Although few fish were examined the results strongly suggest a marked drop in the concentrat of mesenteric fat just at the beginning of maturation and another just before spawning, confirming importance of this depot in maintaining both gonad development and energy needs of the fish dur and after the feeding period (cf. Channon & El Saby, 1932). Hansen (1955) has shown that the herr can cover its energy requirements from its fat reserves based on only 8 months' feeding in a year temperatures of 6-8°C.

Table VIII and Fig. 8 agree with the results of Brandes & Dietrich (1953) and indicate not o the seasonal loss of fat from the alimentary canal during maturation but also the reduction in its c centration with age. A similar situation obtains in the liver (Table X and Fig. 8). Moreover, the val

fat in the alimentary canals and livers of the 1-group fish (Table XIII) are higher than those in the ing fish (Tables VIII and X), thus conforming with the decrease in fat concentration with increase age already observed in these organs from older fish.

The differences between sexes in the weights of non-fatty tissue in the liver during maturation, ecially in the older fish, are very striking (Fig. 12), but it is still uncertain what substances are involved. ar (1957) has shown that in *Salmo salar* there is a large drop in liver glycogen at spawning in females not in males. His values from females, however, are lower than those from males during maturing l spawning, whereas the fat-free weights of herring livers were always higher in females than in males.

Increasing values of the absolute amounts of fat in the developing gonads (p. 25) indicate that the in concentration of fat (Fig. 14) is due, as would be expected, to an increase in the non-fatty tissue ner than to any loss of fat before spawning. Milroy (1908) reported from the results of Secker's analyses imilar cycle in the fat concentrations of the ovaries of Loch Fyne herring, and the same trends at her levels of concentration were observed in North Sea herring by Brandes & Dietrich (1953). ereas Table I of Channon & El Saby (1932) shows that maturity stages are generally correlated with mpling dates, the time trends of the concentration of fat in the gonads in their Fig. 1 are unlike the turity trends shown in Fig. 14, probably because their values were for extracts from bulked gonads, en of different maturities, sampled on the same day. Channon & El Saby did, however, find increases he amounts of fat present during the season similar in relation to maturity to those now reported.

Brandes & Dietrich (1953) found that the concentration of fat in the muscular tissue of the herring highest in the ventral muscle and also high in the dorsal section, decreasing from the region of the sal fin towards the head and tail. The fat values for muscle now given are not, therefore, representative he whole fish, but they refer to the same part of each fish and so are mutually comparable. The fat centration in the muscle was maintained at about the same level during maturation until spawning urred (Fig. 14). Similar findings were reported on the fat of Loch Fyne herring (Milroy, 1906, 1907, 8), Manx herring (Johnstone, 1915, 1918, 1920), North Sea herring (Lovern and Wood, 1937-8) Murman herring (Yudanova 1939). In his work on Manx and Loch Fyne herring, Bruce (1924) erved that the metabolic demands of the gonads were satisfied by the liver fat reserves during matura- and that the muscle fat was not used until spawning; Channon & El Saby (1932) similarly reported rain on the hepatic and intestinal fat during, and on the muscle fat towards the end of, maturation. imilar decrease in muscle fat concentration, occurring slightly earlier than that observed by other kers, was noted by Brandes & Dietrich (1953). Although there is no precise agreement on the stage vhich the muscle begins to lose its fat, the concentration clearly remains at a high level until a later ge in the spawning cycle than it does in the liver and alimentary canal, so providing the economic gastronomic basis of all fisheries for maturing herring.

Bruce (1924) noted that muscle fat reserves increased with age, but his results refer only to herring to five years (four rings). Fig. 14, however, shows that muscle fat remained at a high level in fish with to five rings and decreased in older fish.

Macpherson (1933) observed that the colour of cod liver appeared to be related to its fat content. nough liver colour is related more closely to fat concentration in the liver than in the muscle of the ring, paler livers indicate higher fat reserves in both tissues (Table XI). Liver colour could, therefore, used in the industry to indicate approximately the condition of the herring.

In an autumn-spawning stock of herring the accumulation of the fat reserves depends primarily n feeding and spawning. In spring and early summer when food is abundant and rich in fat, the are all feeding and their muscle fat reserves rapidly build up to a maximum in the early stages of ad maturation (see also Lühmann, 1952; Leim, 1957, 1958; Wood, 1958). Fat in the liver and ientary canal does not accumulate during maturation; in fact as Fig. 8 shows, they are heavily leted of fat at this time, presumably to fulfil the requirements of the gonads. Mesenteric fat appears contribute both to gonad development and to spawning activity. Muscle fat is retained until vning, presumably to supply the energy needs of the fish when its activity is greatest. At this time it is ing rarely, owing to lack of, or intense competition for, food on the spawning ground or to monal or other inhibition of the urge to feed.

SUMMARY

1. Vitamin A and fat were measured in parts of adult herring collected during the north-east Scott summer fisheries of 1953 and 1954, and in young herring from the Moray Firth in December 19 Similar measurements were made on plankton samples collected from the area of the fishery in b years and on gut contents from the adult fish.

2. Vitamin A was not detected in the plankton sampled in 1953 but was found in most of the samp taken in 1954. This difference may have been due to a change in the sampling method. In 1954, vitar A and fat concentration were highest in samples taken in April, and from the north of the area in M with a subsequent decline and then a recovery of fat but not of vitamin A in August. In both ye carotenoids tended to reach higher concentrations in August than in the spring.

3. In the herring, vitamin A concentrations were much higher in the walls of the absorptive pa of the alimentary canal, the pyloric caeca and the intestine, than in the oesophagus and its caecum a the pylorus. Vitamin concentration in any part of the canal was apparently uninfluenced by the s age or maturity of the fish.

4. Vitamin A in the liver increased with increasing age up to 8 rings and decreased in older fi Liver vitamin A reserves increased in maturing fish until about the half-ripe stage in males and almost ripe stage in females and then decreased.

5. Vitamin A, present in 30% of the testes and 52% of the ovaries examined, occurred in simi concentrations in the gonads of both sexes. At all maturity stages, the vitamin was found less frequen in testis than in ovary. Concentration decreased, but the total amount of vitamin A ester and alco in gonads increased, during maturation. Vitamin A aldehyde, now known to occur in herring ovar was not measured.

6. Concentration of vitamin A in the eyes was lower at spawning than at other times.

7. Fat concentration in the absorptive parts of the alimentary canal was higher than in the oesopha and pylorus. The concentration in the whole canal decreased with age and maturity.

8. Fat concentration in the livers decreased with increasing age and maturity. The weight of fat-f liver tissue decreased in maturing males up to stage V and then rose during spawning, but in fema it increased up to stage IV or V, especially in older fish, and then declined again during spawning.

9. In the gonads, the fat concentration decreased during maturation, but was fairly constant relation to age.

10. Fat concentration in the eyes changed little during maturation up to stage V and then decli fairly rapidly in spawning and spent fish.

11. Muscle fat reserves remained high in fish of up to 5 rings but tended to decrease in older fi during maturation they increased slightly and did not fall until spawning actually occurred.

12. The possible contributions of the dietary components of vitamin A and its precursors to vitamin reserves of the herring are considered.

13. Transfers of vitamin A and fat between the various organs of the fish in relation to its matur cycle are discussed.

14. Liver colour could serve the industry as a quick and useful guide to the fatness of a herri

REFERENCES

HMAD, B. *and* DRUMMOND, J. C. 1930. The relative vitamin A value of the body and liver oils of certain fish. *Biochem. J.*, **24**, 27.

CHARACH, A. L., CRUICKSHANK, E. M., HENRY, K. M., KON, S. K., LOVERN, J. A., MOORE, T. *and* MORTON, R. A. 1942. The herring as a source of vitamins A and D. *Brit. med. J.*, **2**, 691.

RRINGTON, E. J. W. 1957. The alimentary canal and digestion. *Brown, M. E. ed. The physiology of fishes. Vol.* 1. *Metabolism.* New York, Academic Press, 1957. 109-161.

AEKKAN, O. R. *and* PROBST, A. 1953. Vitaminer i norsk fisk. I. Nikotinsyre-, riboflavin-, pantotensyre-l vitamin B_{12}- og vitamin A-innholdet i hel fisk og forskjellige organer fra fersk sild (*Clupea harengus*) og makrel, (*Scomber scombrus*). *Fiskeridir. Skr. Teknol. Undersøk.*, **2**, (13), 1.

ANDES, C. H. *and* DIETRICH, R. 1953. Über die Fettverteilung im Körper des Herings. *Veröff. Inst. Meeresforsch. Bremerhaven*, **2**, 109.

UCE, J. R. 1924. Changes in the chemical composition of the tissues of the herring in relation to age and maturity. *Biochem. J.*, **18**, 469.

TLER, C. 1946. Vitamin A and D in fish livers and viscera. *Comm. Fish. Rev.*, **8** (4), 13.

HANNON, H. J. *and* EL SABY, M. K. 1932. Fat metabolism of the herring. I. A preliminary survey. *Biochem. J.*, **26**, 2021.

ISBURY, J. R., MORTON, R. A., SIMPKINS, G. W. *and* LOVERN, J. A. 1938. The distribution of vitamin A and factor A_3. I. *Biochem. J.*, **32**, 118.

SHER, L. R. 1955. Unpublished data.

SHER, L. R. *and* GOLDIE, E. H. 1959. The food of *Meganyctiphanes norvegica* (M. Sars), with an assessment of the contributions of its components to the vitamin A reserves of the animal. *J. mar. biol. Ass. U.K.*, **38**, 291.

SHER, L. R., HARRISON, G. F., KON, S. K., PLACK, P. A., THOMPSON, S. Y. *and* TODD, P. E. E. 1956*a*. An unusual form of vitamin A activity in herring eggs. *Proc. Nutr. Soc.*, **15**, xi.

SHER, L. R. *and* KON, S. K. 1959. Vitamin A in the invertebrates. *Biol. Rev.*, **34**, 1.

SHER, L. R., KON, S. K. *and* THOMPSON, S. Y. 1952. Vitamin A and carotenoids in certain invertebrates. I. Marine Crustacea. *J. mar. biol. Ass. U.K.*, **31**, 229.

— —— —— 1955. Vitamin A and carotenoids in certain invertebrates. III. Euphausiacea. *J. mar. biol. Ass. U.K.*, **34**, 81.

— —— —— 1956*b*. Vitamin A and carotenoids in certain invertebrates. IV. Mollusca: Loricata, Lamellibranchiata and Gastropoda. *J. mar. biol. Ass. U.K.*, **35**, 41.

STER, B. A. 1954. Personal communication.

LLAM, A. E., EL RIDI, M. S. *and* WIMPENNY, R. S. 1939. The seasonal variation in biological composition of certain plankton samples from the North Sea in relation to their content of vitamin A, carotenoids, chlorophyll, and total fatty matter. *J. exp. Biol.*, **16**, 71.

LOVER, R. S. 1956. The environment: the evidence of the plankton. *Rapp. Cons. Explor. Mer*, **143** (1), 22.

— 1957. An ecological survey of the drift-net herring fishery off the north-east coast of Scotland. Part II. The planktonic environment of the herring. *Bull. mar. Ecol.*, **5** (39), 1.

OODWIN, T. W. 1960. Biochemistry of pigments. *Waterman, T. H. ed. The physiology of Crustacea. Vol.* 1. *Metabolism and growth.* New York and London, Academic Press, 1960. 101-140.

RANGAUD, R. *and* MASSONET, R. 1955. Recherches sur la biogénèse de la vitamine A des poissons. I. L'astaxanthine provitamine A pour *Gambusia holbrooki* Grd. *Arch. Sci. physiol.*, **9**, 245.

ANSEN, V. 1955. The food of herring on the Bloden Ground (North Sea) in 1953. *J. Cons. int. Explor. Mer*, **21**, 61.

ARDY, A. C. 1924. The herring in relation to its animate environment. Part I. The food and feeding habits of the herring with special reference to the east coast of England. *Fish. Invest., Lond.*, Ser. 2, **7** (3), 1.

HENTSCHEL, E. 1950. Die Nahrung der Heringslarven. *Helgoländ Wiss. Meeresunters.*, **3**, 59.

HJORT, J. 1910. Report on herring investigations until January 1910. *Publ. Circ. Cons. Explor. Mer*, **53**, 1.

HOAR, W. S. 1957. The gonads and reproduction. *Brown, M. E. ed. The physiology of fishes. Vol.* 1. *Metabolis* New York, Academic Press, 1957. 287-321.

HOLMES, A. D., TRIPP, F. *and* SATTERFIELD, C. H. 1941. Fish liver and body oils. Chemical characteristic physical properties and vitamin content. *Industr. Engng Chem.* (*Industr.*), **33**, 944.

JESPERSEN, P. 1944. Investigations on the food of the herring and the macroplankton in the waters round t Faroes. *Medd. Komm. Havundersøg., Kbh.*, Ser. Plankton, **3** (7), 1.

JOHNSTONE, J. 1915. The fat content of Irish Sea herring. *Proc. Lpool biol. Soc.*, **29**, 216.

—— 1918. The dietetic value of the herring (with special reference to the Manx summer fishery). *Proc. Lpo biol. Soc.*, **32**, 85.

—— 1920. On the dietetic values of herrings and other fishes. *Proc. Lpool biol. Soc.*, **34**, 112.

JUNKER, M. 1952-3. Die Vitamin A-Wirksamkeit von männlichen und weiblichen Heringsgonaden verschi dener Reifegrade. *Arch. FischWiss.*, **4**, 19.

—— 1956. Vitamin A in Fischen, Krebsen und Muscheln. *Arch. FischWiss.*, **7**, 248.

LAMBERTSEN, G. *and* BRAEKKAN, O. R. 1956. Studies on the vitamin A components of fish livers: determinatio and origin. *Biochemical Problems of Lipids*: *Proc. 2nd int. Conf.*, 56.

LEBOUR, M. V. 1923-5. The food of young herring. *J. mar. biol. Ass. U.K.*, **13**, 325.

LEIM, A. H. 1957. Fatness of herring in Canadian Atlantic waters. *Bull. Fish. Res. Bd Can.*, **111**, 177.

—— 1958. Fatness of small herring in the Bay of Fundy. *J. Fish. Res. Bd Can.*, **15**, 1259.

LOVERN, J. A. *and* WOOD, H. 1937-8. Variations in the chemical composition of herring. *J. mar. biol. A U.K.*, **22**, 281.

LÜHMANN, M. 1952. Über die Fettspeicherung bei Ostseeheringen und ihre Beziehung zum Fortpflanzun szyklus. *Kieler Meeresforsch.*, **9**, 213.

LUNDE, G. 1938. Vitaminer i norske naeringsmidler. *Nord. med. Tidskr.*, **15**, 444.

—— 1938-9. Die Vitamine in unseren Nahrungsmitteln und ihre Erhaltung bei der Konservierung. *Vitaminforsch.*, **8**, 97.

LUNDE, G., ASCHEHOUG, V. *and* KRINGSTAD, H. 1937. Fish as a source of vitamins A and D. Herring, brislin and their products. *Industr. Engng Chem.*, **29**, 1171.

MACPHERSON, N. L. 1933. Vitamin A concentration of cod liver oil correlated with age of cod. *Nature, Lon* **132**, 26.

MACPHERSON, N. L. *and* WILSON, A. M. 1935. Technical investigations. A. Vitamin A in liver oils. *Re Newfoundld Fish. Comm.*, **2**, 46.

MARSHALL, S. M., NICHOLLS, A. G. *and* ORR, A. P. 1938. On the growth and feeding of the larval and pos larval stages of the Clyde herring. *J. mar. biol. Ass. U.K.*, **22**, 245.

—— —— —— 1939. On the growth and feeding of young herring in the Clyde. *J. mar. biol. Ass. U.K.*, **23**, 42

MILROY, T. H. 1906. The food value of the herring. *Rep. Fish. Bd Scot.*, **24** (3), 83.

—— 1907. The food value of the herring. *Rep. Fish. Bd Scot.*, **25** (3), 197.

—— 1908. Changes in the chemical composition of the herring during the reproductive period. *Biochem. J* **3**, 366.

MOORE, T. 1957. Vitamin A. pp. xx 645. London: Elsevier.

OGILVIE, H. S. 1934. A preliminary account of the food of the herring in the north-western North Sea. *Rap Cons. Explor. Mer*, **89** (3), 85.

PARRISH, B. B. 1956. Outline of sampling methods used in Scottish fish population studies. *Rapp. Cons. Explo Mer*, **140** (1), 107.

PARRISH, B. B., BAXTER, I. G., MCPHERSON, G. *and* BUCHAN, J. D. 1954. Herring. Scottish Research Work. *Ann. biol., Copenhague,* **10**, 156.

PEREPLETSHIK, R. R. 1951. Content of vitamin A in the liver and other internal organs of commercial fish and porpoises of the Baltic and Azov-Black Sea basins. I. Content of vitamin A in fish of the Baltic Basin. *Akad. Nauk S.S.S.R., Inst. Biokhim. im. A.N. Bakha, Vitaminnye resursy i ikh ispol' zovanie,* **1**, 139. (In Russian).

PLACK, P. A. 1960. Personal communication.

PLACK, P. A., KON, S. K. *and* THOMPSON, S. Y. 1959. Vitamin A_1 aldehyde in the eggs of herring (*Clupea harengus* L.) and other marine teleosts. *Biochem. J.,* **71**, 467.

PLACK, P. A., WOODHEAD, A. D. *and* WOODHEAD, P. M. J. 1961. Vitamin A compounds in the ovaries of the arctic cod, *Gadus morhua* L., during the maturation cycle. *J. mar. biol. Ass. U.K.,* **41**, 617.

RAVAGE, R. E. 1931. The relation between the feeding of the herring off the east coast of England and the plankton of the surrounding waters. *Fish. Invest., Lond.,* Ser. 2, **12** (3), 1.

—— 1937. The food of North Sea herring 1930-1934. *Fish. Invest., Lond.,* Ser. 2, **15** (5), 1.

SCHEUNART, A., CORDUA, R. *and* STAMMER, L. L. 1956. Studien über den Vitamin A-Gehalt von Fischinnereien und Betrachtungen zur Vitamin A-Versorgung. *Ernährungsforsch.,* **1**, 71.

SCHEUNART, A. *and* SCHIEBLICH, M. 1934. Über den Vitamin A-Gehalt des Herings. *Z. Untersuch. Lebensmitt.,* **68**, 409.

SCHMIDT-NIELSEN, S., FLOOD, A. *and* STENE, J. 1933. On the size of the liver of some bone-fishes, their content of fat and vitamin A. *K. norske vidensk. Selsk. Forh.,* **6**, 146.

SCHMIDT-NIELSEN, S. *and* SCHMIDT-NIELSEN, S. 1925. Om A-vitamininholdet hos norsk sild og norske sardiner. *Avh. norske VidenskAkad.,* **8**, 1.

THOMPSON, S. Y., BRAUDE, R., COATES, M. E., COWIE, A. T., GANGULY, J. *and* KON, S. K. 1950. Further studies of the conversion of β-carotene to vitamin A in the intestine. *Brit. J. Nutr.,* **4**, 398.

WAGNER, K. H. 1939. *Vitamin A und β-Carotin des Finn -, Blau - und Spermwals.* pp. 70 Leipzig: Barth.

WILLSTAEDT, H. *and* JENSEN, H. B. 1937. Über den Vitamin A-Gehalt einiger Sorten geraucherter Fische. *Svensk kem. Tidskr.,* **49**, 260.

WOOD, R. J. 1958. Fat cycles of North Sea herring. *J. Cons. int. Explor. Mer,* **23**, 390.

ZUDANOVA, O. N. 1939. The seasonal changes of the fat contents of the Murman herring. *Trans. Knipovich polyar sci. Inst.,* **4**, 95. (In Russian; English summary).

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